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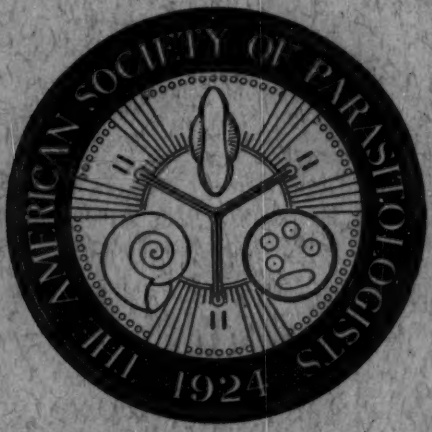

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HAEMOGAMASUS LIPONYSSOIDES HESPERUS, N. SSP., WITH A DISCUSSION OF THE *H. LIPONYSSOIDES* COMPLEX (ACARINA: HAEMOGAMASIDAE)*

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In the course of biological studies on *Haemogamasus liponyssoides* Ewing, 1925, collected in California, the mites were observed to differ from previous descriptions of this species which had been based on material from eastern North America. A comparison with eastern specimens, including types, showed the 2 forms to be very similar but taxonomically distinct. On the basis of the shape of the female sternal plate, the California material is described here as a new subspecies of *H. liponyssoides*. The opportunity to examine a large series and the limitations of previous descriptions of this species have prompted the inclusion of extensive observations on the external morphology of the deutonymph and adults. The descriptions of the egg, larva and protonymph are the first for the species.

There are 4 North American species in the genus *Haemogamasus* Berlese, 1889, with slender, untoothed chelae in both sexes. They are *H. liponyssoides* (2 sspp.), *H. harperi Keegan*, 1951, *H. occidentalis* (Keegan, 1951), and *H. keegani* (Jameson, 1952). They appear to constitute a closely related group within the genus, and are referred to here as the *H. liponyssoides* complex. The cheliceral structure in these species suggests the piercing of intact skin to feed on blood, and this ability has been confirmed for *H. liponyssoides* by Furman (1959). The morphological specialization for haemotophagy approaches that in the Macronyssinae and makes the *H. liponyssoides* complex of particular parasitological interest. The following discussion of the complex is based primarily on the examination of specimens representing 4 of the 5 forms involved.

MATERIALS AND METHODS

The majority of the adults and deutonymphs of the new subspecies of *H. liponyssoides* used for taxonomic study were wild caught. A few specimens of these stages were taken from the F_1 generation in laboratory culture. Eggs, larvae and protonymphs were not obtained in field collections and the descriptions are based on specimens from culture. Culture methods will be described elsewhere.

Specimens of *H. l. liponyssoides* were examined as follows: 2 cotype females, 1 topotype male and 1 topotype female from Ames, Iowa and series or individuals from Illinois, Maryland, New York, Ontario, Canada and Chiapas, Mexico. Slides of *H. occidentalis* from Wayne County, Utah and from several collections in Mono County, California and of *H. keegani* from widely distributed points in California, including one paratype female, were studied. No specimens of *H. harperi* were available for examination.

The principal mounting medium used was C-M medium, a methocellulose preparation

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(Clark and Morashita, 1950). Polyvinyl alcohol with lactophenol was found to aid in distinguishing the plates of the larva and protonymph.

Measurements of the idiosoma were taken from living mites in water mounts. Sufficient water was added to prevent distortion of the mite by the weight of the cover slip, but not enough to permit the mite to tilt onto its side. This technique avoided the progressive distortion caused by the shrinkage of the most commonly used acarological mounting media and permitted accurate measurement of specimens in known biological conditions.

Lengths of the tarsi are exclusive of the ambulacral apparatus. Widths of the tarsi are at the widest points. Length of the tubular portion of the peritreme is from the posterior margin of the stigma to the anterior tip of the tubular portion, in a straight line. All measurements are given in microns.

Haemogamasus liponyssoides hesperus, n. ssp.

To facilitate comparison of the measurements, dimensions of the idiosoma of each of the active stages, including various biological conditions where applicable, are given in table I.

TABLE I. *Dimensions (in microns) of idiosoma of* Haemogamasus liponyssoides hesperus.

Stage and biological condition (10 spec. in each series)	Length		Width	
	Average	Range	Average	Range
Larva	540	518– 551	368	351– 380
Protonymph	589	534– 637	350	313– 378
Deutonymph, unfed	640	616– 675	335	324– 346
Deutonymph, engorged	858	756– 945	495	416– 540
Adult male, starved	842	810– 891	451	421– 481
Adult male, fed	848	821– 891	481	470– 502
Adult female, starved	971	891–1015	541	491– 567
Adult female, engorged and gravid	1272	1161–1405	956	918–1000

Female

Less heavily sclerotized than most mites in genus. Very thickly beset with slender setae. Nearly all setae on unprotected integument of idiosoma barbed, except for metasternal setae and setae covering region between genitoventral and anal plates. Dorsal plate with barbed setae near anterior margin and sometimes near lateral or posterior margins (usual pair of apical setae always smooth). All other plates, gnathosoma and legs with smooth setae only. Barbed setae bearing 1 to 3 barbs on one side, those away from midline of body curving toward it and having barbs on outside of curvature (fig. 2a).

Dorsum: Dorsal plate covering all or nearly all dorsal surface of starved mite; sculptured throughout, but very indistinctly on anterior half. Usual pair of apical setae 90 long, distinctly heavier than nearby setae. Setae in middle of plate subequal, about 55 long, on margin of plate and sides of idiosoma slightly longer, on posterior border of idiosoma 75 long.

Venter: Base of tritosternum with minute marginal or submarginal spines; laciniae fimbriated and each bearing ventrally near base a transverse row of small spines. Sternal plate (fig. 3) invaginated posteriorly to about level of 3d pair of sternal setae (of 42 specimens none with plate invaginated beyond one-sixth the distance from 3d to 2d pairs of sternal setae); invagination varies from narrow medial emargination to broad, shallow trough; posterior margin of plate often with distinctly irregular outline. In addition to 2 pairs of pores on sternal plate, a 3d pair, nearly round in outline, located anterior and medial to bases of metasternal setae. Genitoventral plate only slightly expanded behind coxae IV, not reaching posterior half of opisthosoma. In 42 specimens, 22 to 32 accessory genitoventral setae with mean of 26.5. Anal plate about twice as long as wide, rounded anteriorly; anal opening in middle of plate; scobination on posterior tip reaching, on sides of plate, anterior to unpaired usual anal seta. Seven accessory anal setae (occasional specimen with 6 or 8), 3 on anterior margin of plate and 2 pairs between anterior margin and anal opening. Metapodal plates distinctly elongate; long axis anteroposterior; 45–60 long, 12–16 wide. Endopodal plates very slender and lightly sclerotized; anterior ends free; posterior ends contiguous with and appearing to fuse with foveae of coxae IV. Tubular portion of peritreme extends parallel to posterior one-sixth to one-half of coxa II; length 207–261 with mean of 235. Anterior end of peritremal plate fusing with dorsal plate; posterior end fusing with fovea of coxa IV. Setae of unprotected integument of opisthosoma subequal, about 70 long, except for one pair of caudal setae, 110 long. Tarsal lengths and widths: I 262 × 49, II 201 × 52, III 246 × 49, IV 372 × 50. Exceptionally long seta near middle of dorsal surface of tarsus IV, 220 long.

Gnathosoma: Deutosternal groove with 11 or 12, occasionally 10 or 13, rows of teeth;

1 to 13 teeth per row. Palpus with broad, flattened seta on ventral face of trochanter; small seta with rounded hyaline tip on medial face of genu. Tectum (fig. 4d) with broad tip; fimbriae on tip and on sides for distal one-fourth or less; fimbriae long and slender; some fimbriae branched, especially on tip. Chelicerae (fig. 1d) with long, slender chelae. Fixed chela with tip pointed and curving ventrally; minute pilus dentilus near beginning of distal curvature and slender basal seta. Movable chela distinctly heavier than fixed chela; tip rounded; dorsomedial groove (to accommodate fixed arm) running most of length of chela.

Male

In general, setae shorter than corresponding setae of female. Fimbriated setae of same type and with same distribution as in female, usually covering lateral thirds of expanded portion of holoventral plate.

Dorsum: Dorsal plate covering all or nearly all of dorsal surface, sculptured as in female. Usual apical setae 70 long. Setae in middle of plate about 43 long.

Venter: Tritosternum as in female. Holoventral plate covering most of ventral surface of opisthosoma; sides curving anteriorly lateral to coxae IV; anal area not distinctly demarcated. Distance of sternal, metasternal and usual genitoventral setae from lateral margins of plate equal to, or greater than, diameter of their trichophores. Three pairs of pores on holoventral plate. Endopodal plates absent. Pair of long caudal setae, 80 long. Peritreme as in female; length of tubular portion 156–201 with mean of 182. Tarsal lengths and widths: I 207 × 43, II 156 × 46, III 190 × 39, IV 287 × 43. Femur, genu and tibia of leg II each with regularly tapering, distinctly thickened ventral seta. Tarsus II with thickened ventral seta (fig. 2b) greatly swollen on one side of base. Tarsus IV with long dorsal seta, 177 long.

Gnathosoma: Deutosternal groove as in female, but with somewhat more teeth per row (4–14). Deutosternal groove bordered anteriorly by 3 depressions on each side (fig. 2c); 1 to 8 basally inflated teeth on floor of each depression. Palpus as in female. Tectum (fig. 4e) similar to female but with small barblike fimbriae extending back on sides, occasionally to base of tectum. Chelicerae (fig. 1e) with long, slender, unbranched, untoothed chelae. Fixed chela rounded and bent medially near tip to terminate in flat medial surface; short, but rather stout and porrect, pilus dentilus on medial surface two-thirds of length from base; slender seta arising ventrally at base. Movable chela longer than fixed chela, curving dorsally near tip; tip surrounded ventrally and laterally by membranous bursa. Subtriangular shield covering bases of chelae medially.

Deutonymph (*Fig. 8*)

Density of setation as in adults. Fimbriated setae of same type and with same distribution as in adults.

Dorsum: Dorsal plate without lateral invaginations; plate narrow (300 at greatest diameter), leaving margin of unprotected integument even in unfed specimens; sculpturing as in adults. Usual apical setae distinctly thicker than nearby setae but only slightly longer, about 60 long. Setae in middle of plate of more variable length than in adults, about 43 long.

Venter: Tritosternum as in adults. Presternal area sculptured; posteriorly directed spines along sutures. Ventral plate sculptured; sternal and metasternal setae (in occasional specimens also genitoventral setae) on plate; setae on lateral margins of plate except 1st pair of sternal setae; 3 pairs of pores on plate, with posterior 2 pairs on lateral margins; sides of plate subparallel back to 3d pair of sternal setae; tip of plate varying from acuminate to broadly rounded, frequently irregular in outline. Genitoventral setae normally flanking posterior tip of ventral plate. Two pairs of small irregular platelets posterior to tip of ventral plate; each platelet typically bearing a single seta, but 1 or more may be bare. Anal plate as in female but with 3 accessory setae, all on anterior margin of plate. Metapodal plates small, irregular in shape, from nearly round to distinctly elongate, with long axis almost lateral in orientation. No visible endopodal plates. Tubular portion of peritreme extending parallel to posterior one-half to all of coxa II; length 213–255 with mean of 229. Peritremal plate less developed than in adults; anterior and posterior ends not fusing with other sclerites. Pair of long caudal setae, 88 long. Relative lengths of legs from longer to shorter: IV, I, III, II; all coxae sculptured. Tarsus IV with long dorsal seta, 140 long.

Gnathosoma: Deutosternal groove as in female, but with generally fewer teeth per row (1–9). Palpus as in adults. Tectum (fig. 4c) with rounded tip; all fimbriae on tip; fimbriae shorter and more branched than in adults. Chelicerae (fig. 1c) as in female except for smaller size.

Protonymph (*Fig. 7*)

Idiosoma widest near middle. Integument lightly sclerotized; plates not easily distinguishable, without sculpturing. Idiosoma lacking dense setation of later stages; rather conspicuous long seta on posterior margin.

Dorsum: Two dorsal plates (fig. 5), partially contiguous or nearly so, covering most of dorsal surface. Anterior plate largest, covering most of podosoma, tapering anteriorly. Posterior plate wider than long. Plates and unarmed dorsal integument moderately clothed with setae, about 35 pairs. Setae about 75 long, except 7 to 10 pairs near posterior margin of opisthosoma, 90–100 long. Setae smooth except for posterior setae sometimes having minute fimbriae.

Venter: Tritosternum with fimbriae on laciniae and sides of base. Ventral plate broad with broadly rounded posterior margin, widest between second and third pairs of setae; 3 pairs of setae and 2 pairs of pores on plate. Anal plate broadly rounded anteriorly; 3 usual anal setae present; posterior scobination present. Unarmed portion of venter with about 9 pairs of smooth setae of moderate length, located posterior to coxae IV; 1 pair of long (90–100) posterior setae on venter. Peritreme 26–33 long, with characteristic shape; stigmal opening and peritremal plate not visible. Legs I and IV and legs II and III of approximately equal length; legs I and IV longest; leg II stoutest. With fewer setae on legs than in later stages, all smooth. Long dorsal seta on tarsus IV, 120 long. All tarsi with well developed caruncles and claws.

Gnathosoma: One pair of gnathosomal and 3 pairs of hypostomal setae, arranged as in later stages. Deutosternal groove as in later stages. Palpus with seta with hyaline tip on genu and usual two-tined seta on tarsus, but without inflated seta on trochanter. All gnathosomal setae smooth. Tectum (fig. 4b) with 12 to 20 very short distal fimbriae, some branched. Chelicerae (fig. 1b) stouter than in later stages; chelae short, bluntly pointed, untoothed, without setae; arc of microsetae at base of movable chela.

Larva (*Fig. 6*)

Idiosoma widest near posterior margin which is very broadly rounded. Integument very weakly sclerotized; plates only faintly demarcated, without sculpturing. Idiosoma sparsely setate. Barbed setae on legs and palpi with numerous barbs not restricted to one side. Conspicuous long setae on posterior margin of idiosoma.

Dorsum: Some indication of dorsal plate, but shape not determined. Dorsum with 16 pairs of setae, all usually smooth; anterior pair and 2 submedian pairs shortest; 6 posterior pairs longest, 100 long.

Venter: Tritosternum with base and laciniae fimbriated. Ventral plate approximately as in fig. 6; 3 pairs of setae on plate; no pores. Anal plate with 3 usual anal setae, scobinate posteriorly. Unarmed portion of venter with 6 pairs of smooth setae; first pair of setae posterior to ventral plate very small, 15 long; 2 pairs of long posterior setae similar to those on dorsum. Stigmal opening, peritreme and peritremal plate lacking. Leg I longer than legs II and III, the latter of equal length; leg II stouter than others. Majority of dorsal setae on legs I and II and some dorsal setae on leg III barbed; few barbed setae ventrally on all legs. All legs with well developed caruncles and claws.

Gnathosoma: One basal pair and 1 distal pair of smooth hypostomal setae; gnathosomal setae absent. Deutosternal groove with up to 10 rows of teeth. Palpus with hyaline-tipped seta on genu and usual two-tined seta on tarsus. Most of dorsal setae on palpus barbed. Tectum (fig. 4a) acuminate, with numerous minute teeth on distal margin and scattered teeth on dorsal surface. Chelicerae (fig. 1a) short and stout; short, broad chelae, each with 2 or 3 distal teeth; arc of microsetae at base of movable chela.

Egg

Length 454–540, mean 500; width 329–373, mean 348. Elliptical. White with yellowish cast. Shell 2–3.5 thick; transparent with smooth, unornamented surface. Developing embryo readily visible through shell.

Other Data

Type host: Microtus californicus (Peale)

Type locality: Strawberry Canyon, Berkeley, Alameda County, California.

Types: Holotype female, allotype male and 7 females, 5 males and 7 deutonymphs in the paratype series taken by the writer from nests of *M. californicus,* from Strawberry Canyon in the Berkeley hills, Alameda County, California, from September 1957 through May 1958. Also designated as paratypes are 24 females, 5 males and 9 deutonymphs collected by H. E. Stark from nests of *M. californicus,* from near San Andreas Lake, San Mateo County, California, from November 1957 through January 1958. Two males, 3 females and 2 deutonymphs from among the paratypes have been placed in the mite collection of the University of California at Berkeley. The holotype, allotype and remaining paratypes will be deposited with the United States National Museum.

Other hosts and localities: In addition to the type series, the author has identified specimens as *H. l. hesperus* from the following hosts and California counties: *M. californicus* from

Contra Costa and Marin Counties, nests of *Neotoma fuscipes* Baird from Contra Costa and San Mateo Counties, *Clethrionomys occidentalis* (Merriam) from Plumas County, *Neurotrichus gibbsi* Baird from Plumas County and *Scapanus latimanus* (Bachman) from Contra Costa County. The mites from Plumas County are in the E. W. Jameson Collection, and the other specimens are in the collection of the University of California at Berkeley. From his remarks and the figure of the female sternal plate, the mites from Plumas County discussed by Jameson (1952) as *H. l. liponyssoides* undoubtedly are *H. l. hesperus.*

The subspecific name is taken from the Greek word *hesperos* meaning western and refers to the geographic range in North America, relative to that of *H. l. liponyssoides.*

The subspecies is established on the basis of the degree of invagination of the posterior margin of the female sternal plate. This plate is invaginated in *H. l. liponyssoides* to a point halfway between the 2d and 3d pairs of sternal setae, in *H. l. hesperus* to the 3d pair of sternal setae. The redescription of *H. liponyssoides* by Keegan (1951) attributes other characters to this form which are different from those possessed by *H. l. hesperus.* However, examination by the present author of the type specimens and other material of *H. liponyssoides* from eastern North America revealed that *H. l. liponyssoides* and *H. l. hesperus* agree in all the characters considered, except for the degree of sternal plate invagination described above. Thus, females, males and deutonymphs of both subspecies possess a distal seta on the fixed chela, widely distributed barbed setae on the unprotected idiosoma and fimbriae on the laciniae of the tritosternum.

The known host range of *H. l. hesperus* includes 3 genera of rodents and 2 genera of insectivores. *H. l. liponyssoides* has been recovered from a great variety of rodents and insectivores, and undoubtedly the hosts known for *H. l. hesperus* will increase rapidly with continued collecting.

H. l. hesperus has been collected in several counties bordering San Francisco and San Pablo Bays along the coast of California. It also has been taken in Plumas County which straddles the Sierra Nevada Mountains. *H. l. liponyssoides* is found over much of eastern North America and has been recorded as far west as Iowa and from Chiapas, Mexico. There remains the whole area from the eastern Great Plains to the Sierras from which collection of the species has not been recorded.

The *H. liponyssoides* Complex

In addition to *H. liponyssoides, H. harperi, H. keegani* and *H. occidentalis,* there are 2 other species in the genus *Haemogamasus* with long slender chelae in the female. These are *H. hirsutus* Berlese, 1889, found in Europe and *H. quadrisetatus* Vitzthum, 1926, recorded from Asia. Although these species may have had a common origin with the *H. liponyssoides* complex, they are distinctly removed from it. *H. hirsutus* has the chelicerae of the male highly modified for sperm transfer. *H. quadrisetatus* is known only from the female, but this has a number of distinctive features including widely distributed barbed setae on the legs and gnathosoma, characteristically shaped dorsal and genitoventral plates and 4 extremely long setae on the posterior margin of the idiosoma.

In addition to their cheliceral structure, the species of the *H. liponyssoides* complex may be characterized by several features. The deutonymphs lack lateral invaginations in the dorsal plate. There normally are 7 accessory setae on the female anal plate, arranged in a characteristic pattern. All setae on the legs, gnathosoma and female sternal plate are smooth.

H. occidentalis was described originally as a subspecies of *H. liponyssoides*. Strandtmann and Wharton (1958) gave *H. occidentalis* species rank without comment as to their reasons for doing so. The present writer also considers this form to be a distinct species. It differs morphologically from the other species in the complex in having stouter chelicerae, male chelicerae more modified for sperm transfer and a regularly tapering, rather than a basally swollen, thickened seta on tarsus II of the male. *H. occidentalis* occurs in the western part of North America and its range overlaps the ranges of *H. l. hesperus* and *H. keegani*. In common with the other species in the complex, it has a wide host range, and there is little likelihood of isolation through host association.

As Jameson (1952) has pointed out, *H. harperi* and *H. keegani* appear to be very closely related. They are separated primarily by the presence of a small number of accessory setae on the female sternal plate of *H. harperi*. The reliability of this characteristic is placed in doubt by the work of Asanuma (1951), who described a species of *Haemogamasus* in which the accessory setae on the female sternal plate varied from 0 to 6. The short tubular portion of the peritreme, small length-width ratios of the tarsi and smaller number of accessory genitoventral setae set *H. harperi* and *H. keegani* apart from *H. liponyssoides* and *H. occidentalis*. *H. keegani* is found in the western United States and *H. harperi* in the southeastern United States. The combination of morphological similarity and allopatric distribution suggests that these 2 forms represent subspecies of a single species; however, a change in rank should not be made without a detailed comparison of extensive series representing each of the 2 forms.

Unilaterally barbed setae have much the same distribution in *H. occidentalis* and *H. keegani* as they do in *H. liponyssoides*. The toothed depressions (fig. 2c) which border the deutosternal groove in males of *H. liponyssoides* are entirely absent in *H. keegani*. Males of *H. occidentalis* have smaller scattered teeth in this region but no depressions.

Key to Females of the *H. liponyssoides* Complex

1. With accessory setae on sternal plate.....*harperi*
   Without accessory setae on sternal plate.....2
2. With 11 to 15 accessory setae on genitoventral plate.....*keegani*
   With 19 or more accessory genitoventral setae.....3
3. Tectum with distal fimbriae long and lateral fimbriae, when present, short and barblike; deutosternal groove broad, usually with 8 or more teeth in each of the 4 anterior rows *occidentalis*
   Tectum with long fimbriae laterally as well as distally; deutosternal groove narrow, with 1 to 8 teeth in each of the 4 anterior rows.....*liponyssoides* 4
4. Sternal plate invaginated posteriorly to about halfway between second and third pairs of sternal setae.....*l. liponyssoides*
   Sternal plate invaginated only to about level of third pair of sternal setae .....*l. hesperus*

Key to Males of the *H. liponyssoides* Complex

1. With regularly tapering thickened seta on ventral surface of tarsus II.....*occidentalis*
   With ventral seta on tarsus II swollen on one side and with a slender distal portion.....2
2. Holoventral plate not curving anteriorly lateral to coxae IV; anal region of plate long and narrow.....*harperi* or *keegani*
   Holoventral plate curving anteriorly lateral to coxae IV; plate with broad, poorly demarcated anal region.....*liponyssoides* sspp.

No reliable characters are known for separating the males of *H. harperi* from *H. keegani* or *H. l. liponyssoides* from *H. l. hesperus*.

SUMMARY

Haemogamasus liponyssoides hesperus is described as a new subspecies of parasitic mite from California. All the stages in the life cycle, egg, larva, protonymph, deutonymph and adult male and female, are described. *H. liponyssoides* and 3 other species of the genus *Haemogamasus*, with slender, untoothed chelae, are discussed taxonomically as the *H. liponyssoides* complex. Keys are given to the adults of this complex.

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The writer wishes to thank Prof. Deane P. Furman, under whose guidance this study was conducted, E. P. Catts who inked in the plates, H. E. Stark who collected the mites from San Mateo County, Dr. E. W. Jameson for the loan of slides, and Dr. E. W. Baker who loaned the type slides of *H. l. liponyssoides* from the collection of the U. S. National Museum. This study was supported in part by research grant E-1509 from the National Institute of Allergy and Infectious Diseases, of the National Institutes of Health, Public Health Service.

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EXPLANATION OF PLATES

PLATE I

Haemogamasus liponyssoides hesperus. FIGURE 1. Chelicerae of: a, larva; b, protonymph; c, deutonymph; d, female; e, male (a is a ventral view, b–e are medial views). FIGURE 2. a, barbed seta from idiosoma of adult; b, basally enlarged seta on tarsus II of male; c, portion of male gnathosoma, ventral view showing deutosternal groove and toothed depressions bordering it. FIGURE 3. Sternal plate of female. FIGURE 4. Tecta of: a, larva; b, protonymph; c, deutonymph; d, female; e, male. FIGURE 5. Idiosoma of protonymph, dorsal view showing dorsal plates.

PLATE II

Haemogamasus liponyssoides hesperus. FIGURE 6. Larva. FIGURE 7. Protonymph. FIGURE 8. Deutonymph, sculpturing omitted except in presternal region.

PLATE I

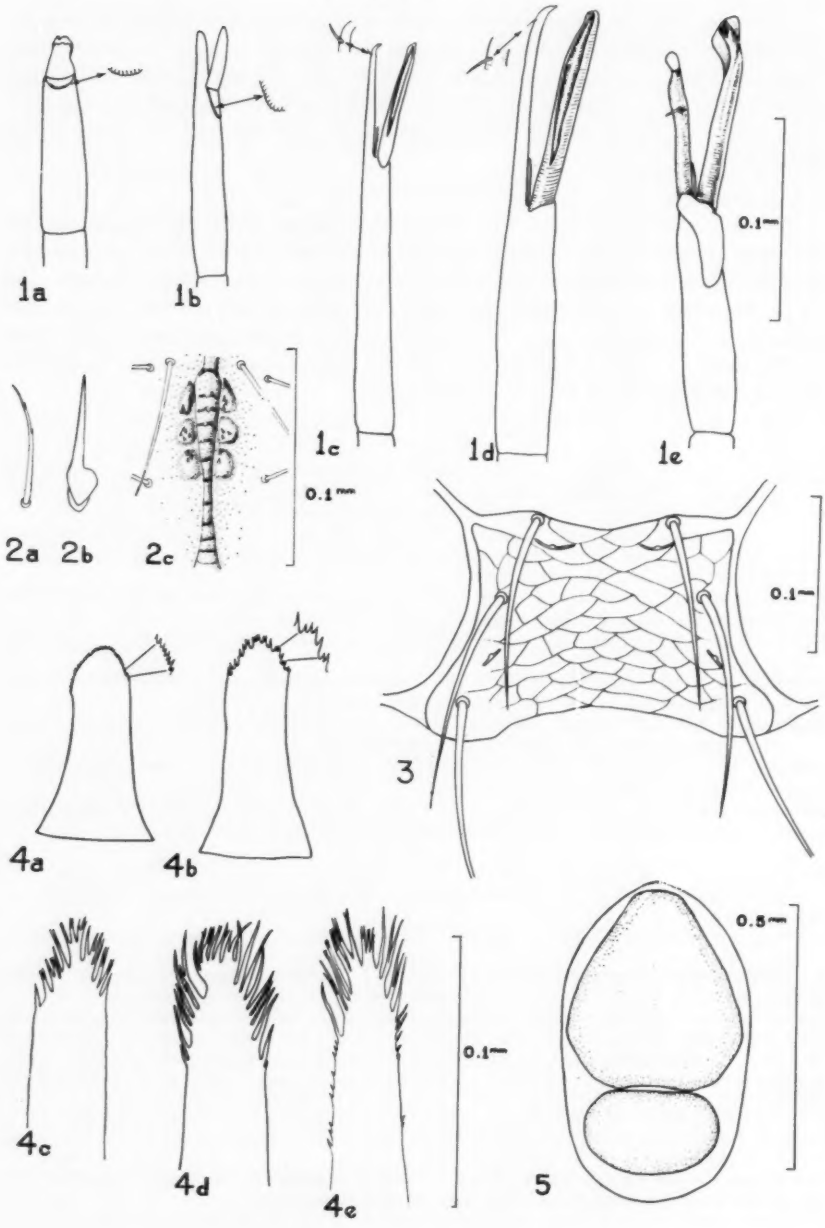
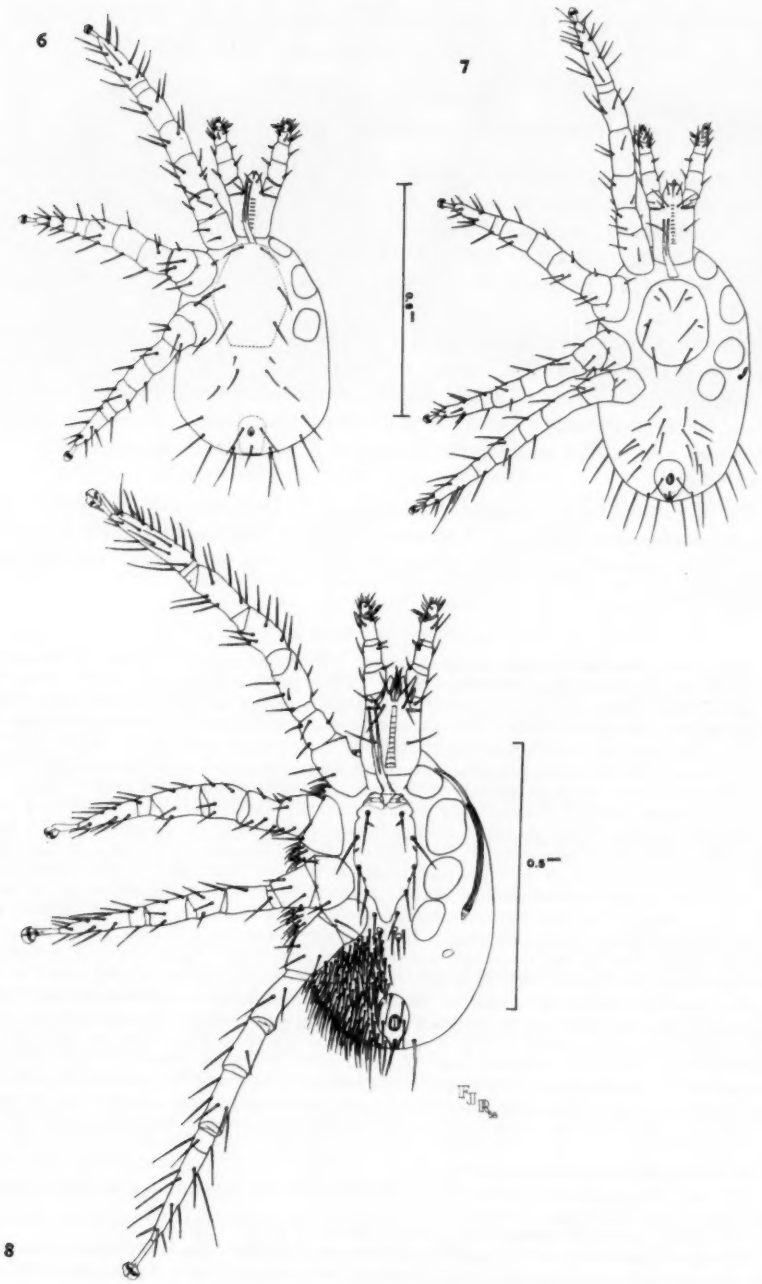


PLATE II



BIOLOGICAL STUDIES ON *HAEMOGAMASUS LIPONYSSOIDES* EWING (ACARINA: HAEMOGAMASIDAE)*

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Haemogamasus liponyssoides Ewing, 1925, is a parasite of rodents and insectivores, widely distributed in North America. It is one of 4 closely related North American species in the genus *Haemogamasus* Berlese, 1889, in which the chelicerae have long, slender, untoothed chelae similar to those found in macronyssine mites. The majority of the species in this large genus have heavy, toothed chelae of a more generalized type.

Furman (1959a) described *H. liponyssoides* as penetrating the intact or abraded skin and sucking blood from several rodent hosts in the laboratory. He found *Haemogamasus ambulans* (Thorell, 1872) with heavy, toothed chelae to be a generalized feeder, accepting a variety of substances that might be available to it in a rodent nest. A detailed study of the biology of *H. ambulans* was reported by Furman (1959b).

The mites used for the present studies belong to the subspecies *H. liponyssoides hesperus* Radovsky, 1960 (see Radovsky, 1960). The material studied by Furman (1959a) also belongs in this subspecies, which thus far has been recorded only in California.

MATERIALS AND METHODS

Mites were obtained from nests of *Microtus californicus* (Peale), taken in Strawberry Canyon in the Berkeley hills, Alameda County, California. Glass tubes in which mites were kept were 3 inches long with an inside diameter of slightly more than 0.25 inch, and were plugged at each end with absorbent cotton. The cotton plug in one end of each tube was moistened with distilled water, and the tubes were kept in humidity chambers at about 20 C (19–23 C). The relative humidity in the tubes, measured with cobalt thiocyanate papers (Solomon, 1957), ranged from 95 percent over most of the tube to 100 percent near the moistened plug. Plastic containers of 2 types, available commercially as food receptacles, also were used for holding mites. The square containers (fig. 1), referred to as boxes, were 4 inches in diameter and 2.5 inches deep. The round containers, referred to as dishes, were 3 inches in diameter and 1.75 inches deep. The central portion of the lid of each container was replaced with 100-mesh nylon screen.

Mites fed extracted blood were maintained in tubes. Blood was placed on one of the cotton plugs in the tube, or the mites were transferred for 30-minute feeding periods to a screen-topped plastic dish containing a drop of blood. Except where otherwise indicated, blood was taken from laboratory mice by heart puncture and mites were fed daily. Blood not used immediately after extraction was treated with .02 ml of 1 percent sodium heparin per ml and stored at 4 C.

The plastic containers were used for the maintenance of mites on suckling laboratory mice. Dishes were used for maintaining individual mites and boxes primarily for mass cultures. Four balls of absorbent cotton about 1 inch in diameter were placed in the corners of each box and 1 in each dish. Sterilized material from *Microtus* nests, consisting mainly of fine, dry grass, was used to fill the remaining space in the containers to the height of the cotton balls. In a few experiments, cut dried grass was substituted for the nest material and appeared to be equally satisfactory. The containers were held in a well-ventilated room with temperatures of 18–28 C,

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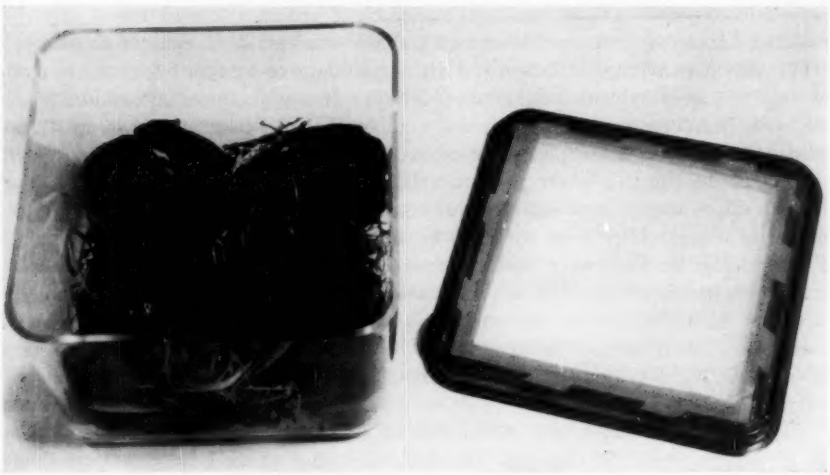

FIGURE 1. Plastic box used for mass culturing. The screened lid is removed and cotton balls, nest material, and a suckling mouse are visible inside the box.

and the cotton was kept wet with distilled water. Suckling mice from 1 to 7 days old were used in most experiments; mice up to 21 days old were used in feeding large cultures. Sucklings

TABLE I. *Variations in length of the non-feeding stages of* Haemogamasus liponyssoides.*

Stage	No. of mites tested	Days 1	2	3	4	5
		Percent mites hatching or moulting				
Egg**	39	26	13	15	33	13
Egg***	29	0	0	21	76	3
Larva	92	86	13	1	0	0
Protonymph	80	7	83	9	1	0

* In tubes at about 20 C and at 95 to 100 percent relative humidity.
** From females feeding irregularly on stored heparinized blood.
*** From females consistently engorging in mass cultures on suckling mice.

were exposed to the mites for 24 to 48 hours and then placed with a nursing female for at least 24 hours before being re-exposed.

RESULTS

Life History

Haemogamasus liponyssoides has 5 stages: egg, larva, protonymph, deutonymph, and adult male and female. Table I gives the variations in length of the first 3 stages at about 20 C. Females in mass cultures, where feeding was optimum, deposited unembryonated eggs, and the duration of the egg stage was consistently about 4 days. However, feeding on stored blood was poor, and resulted in a reduced reproductive rate and the frequent retention of eggs until late in their embryonic development. Under these conditions, eggs hatched within as short a period as 15 hours. The larvae and protonymphs did not require food in order to moult, and there was little variation in the length of these stages. Larvae usually moulted in 1 day or less and protonymphs in about 2 days.

The deutonymphs required repeated engorgement in order to reach the adult stage. When conditions resulted in poor feeding, mites remained in the deutonymphal stage for up to 2 months. The maximum free feeding of individually ob-

served deutonymphs was on freshly extracted blood in screen-topped dishes. Newly moulted deutonymphs fed in this manner and held at about 20 C, moulted as follows: 5 to males in an average of 7 days and with a minimum of 5 days; 5 to females in an average of 9 days and with a minimum of 7 days. In mass cultures on suckling mice, the cycle from egg to egg regularly was completed in 3 weeks, and on at least one occasion several mites completed the cycle in 2 weeks.

Eggs. In cultures where nest material was made available to the mites, the smooth elliptical eggs were deposited between adjacent blades of grass or in crevices in bits of wood. They were not fixed by any secretion and were easily dislodged. Four pairs of leg buds were visible through the egg shell at an early stage in the embryonic development. The 4th pair of legs was no longer apparent later in the development of the embryo, but was visible again through the opisthosomal integument of the newly hatched hexapod larva. Camin (1953) observed the same series of events in the development of *Ophionyssus natricis* (Gervais, 1844), a dermanyssid mite.

Larvae and protonymphs. At the time of hatching, a longitudinal split appeared on the ventral surface of the egg. The final extrication of the larva took only about 15 seconds and was accomplished by active movements of the palpi and legs. Newly hatched larvae were immediately active and moved about if stimulated. The normal gait of the larvae was slow and lumbering, but they moved in short bursts of speed when touched by other mites or by an instrument. Protonymphs ran somewhat more rapidly. Both larvae and protonymphs were white and poorly sclerotized. These stages, particularly the protonymphs, took free blood when it was offered. However, feeding on blood did not affect the length of stage, and they failed to feed in mass cultures which simulated conditions found in nature.

Deutonymphs and adults. The deutonymphs and adults moved rapidly. They were more heavily sclerotized than the earlier stages and, except when newly moulted, had a yellowish cuticulum. When engorged, they were bright red or black, depending on the proportion of old blood in the gut. Deutonymphs and females became greatly swollen when feeding, but males increased only slightly in size.

Deutonymphs moulted to adults with as few as 3 engorgements, but usually more were required. More food was required by deutonymphs of the female than those of the male in order to moult. Five deutonymphs moulting to males engorged on an average of 4.0 occasions and 5 moulting to females on an average of 6.7 occasions. Under conditions where feeding was poor, nearly all the mites that moulted produced males. At about the time the adult characters first became visible through the cuticulum, deutonymphs required no additional food in order to moult and became relatively inactive. They moved about slowly and did not react readily to stimuli. This period lasted for 1 to 3 days before the moult.

Behavior during copulation agreed with the detailed description given by Michael (1892) for *Haemogamasus hirsutus* Berlese, 1889. The copulatory position was frequently maintained for periods of 0.5 to 1 hour. However, dissection of the female from a mating pair forcibly separated after 7 minutes showed that the contents of the spermatophore had been introduced. Both males and females mated successfully within 24 hours after the deutonymphal moult. Insemination of 2 females by a single male was observed. Inseminated females repulsed attempts by males to mate, and oviposition was observed in some instances for 50 days after copulation.

Feeding at least once daily was necessary to maintain a high rate of oviposition. The maximum rates observed were about 2 eggs a day, sustained for a 10-day period by each of 2 females kept on suckling mice. The absence of parthenogenetic reproduction was demonstrated by maintaining 7 virgin females on suckling mice for 14 to 37 days without the recovery of young, while inseminated controls produced eggs regularly. Males and females appeared to be produced in about equal proportions.

Feeding Behavior

Extracted blood from the laboratory mouse, the laboratory rat and *Microtus californicus* were equally acceptable to the mites. Sucklings of the laboratory mouse, *M. californicus,* and *Reithrodontomys megalotis* Baird were utilized as hosts, and deutonymphs, males and females held separately were each able to pierce the skin and feed on blood. Mites also fed when given continual access to an adult *M. californicus* in a nest box provided with a high humidity. Three female mites, placed on the writer's forearm in a feeding chamber screened on the free end, failed to feed during a 3-hour period. Although cultures with many individuals were handled frequently, mites did not seem attracted to human skin and feeding was not observed. Four deutonymphs, 5 males, and 1 female were offered parts of freshly killed soldier flies or injured flea larvae daily for 10 days without evident feeding. There was no indication of cannibalism, such as Furman (1959a) observed in *H. ambulans.*

Feeding on extracted blood. When heparinized blood, used for up to 2 weeks after extraction, was placed on the cotton plugs of their tubes, mites fed poorly and lacked the engorged appearance frequently seen in wild-caught mites. Deutonymphs moulted only after several weeks if at all, and females reproduced infrequently and irregularly. With the introduction of fresh lots of heparinized blood, an improvement in feeding was noted, and this was enhanced by the transfer of mites to clean tubes; however, the improvement lasted only 1 or 2 days. In one test, feeding of freshly extracted blood to deutonymphs in different types of containers resulted in the engorgement of none of 7 mites in tubes and 8 of 12 mites in screen-topped plastic dishes. Regular feeding of either freshly extracted blood in tubes or stored blood in dishes failed to result in consistent engorgement or moulting of deutonymphs. However, mites engorged thoroughly when they occasionally were entrapped in blood under any of these conditions. Intentional forced feeding with a low mortality rate was achieved by immersing mites in blood for 10 minutes and then carefully washing them in distilled water.

The above experiments suggested that a combination of fresh blood and circulating air might provide consistent engorgement, and this was obtained in the experiment summarized in table II. Free feeding, that is without immersion, at daily intervals resulted in 80 percent of the deutonymphs moulting in 7 to 11 days. That maximum engorgement still was not obtained in free feeding was indicated by the failure of mites fed at more than 24-hour intervals to moult unless fed by immersion. The 16 deutonymphs failing to moult in this experiment were force fed on each of the 2 days immediately following. Four did not survive handling, but the other 12 moulted within 3 days after the last immersion. In other experiments, the use of fresh blood and open containers improved feeding by adult mites.

Feeding on suckling mice. Mites given access to a host under a great variety of conditions rarely fed when exposure was for several hours or less. Feeding improved when continual access to suckling mice was provided in screen-topped

plastic containers with wet cotton and nest material. Excellent results always were

TABLE II. *Results of feeding newly moulted deutonymphs of* Haemogamasus liponyssoides *on fresh blood in open dishes over a 13-day period.*

Interval in hours between feedings	Feeding method	No. of mites tested	Percent moulting
24	free feeding	10	80
48	free feeding	9	0
72	free feeding	5	0
72	forced feeding by immersion	3	100

obtained with 5 or more inseminated females in each container; one culture started with 10 gravid females increased to nearly 600 mites in 21 days. In containers with numbers of deutonymphs or males but no females, feeding was often poor. Although some individually held mites produced 2 eggs a day or completed the deutonymphal stage in 7 days, others fed poorly or not at all. Occasional changing of nest material and increased ventilation improved feeding of individually held mites.

Individual mites never produced more than a single wound on suckling rodents during a 24-hour period. In mass cultures, several hundred mites in the feeding stages usually produced only 1 or 2 wounds on suckling laboratory mice, although nearly all the mites fed. Numbers of mites were seen feeding on blood flowing from a single wound. All parts of the mice were attacked by mites, but areas with delicate skin, such as parts of the head and feet, were most favored. There was a tendency to feed in or around natural orifices, particularly the anal and genital openings.

Effects on the Host

The lesion produced on suckling rodents appeared as a small puncture, usually surrounded by an extensive purplish zone of discoloration caused by internal hemorrhaging, and frequently with a copious external flow of blood (fig. 2). The discoloration covered a greater area when numbers of mites had fed at the wound. With respect to feeding by individual mites, females produced more obvious lesions than males or deutonymphs.

Laboratory mice from 1 to 7 days old were able to survive 48 to 72 hours in cultures with only 1 or a few mites. With 300 to 500 feeding mites, mice were usually dead after exposure for 24 hours. Lesions healed rapidly on surviving mice.

Longevity of Fed and Starved Mites

Females were maintained on heparinized blood up to 105 days and males up to 75 days; however, observations were terminated with some mites of each sex still living.

The ability to survive in the absence of a host is of particular importance to a nest-inhabiting parasite. Unless the non-feeding stages of *H. liponyssoides* have a markedly greater duration at low temperatures, mites may exist for long periods only as deutonymphs or adults. The survival times in days for mites kept in tubes without food were as follows: 5 females, 33, 33, 40, 47, 69; 5 males, 33, 33, 34, 38, 47; 4 engorged deutonymphs, 24, 29, 37, 44; 4 unfed deutonymphs, 12, 14, 24, 26.

Humidity Requirements

Eggs, larvae, and protonymphs held at room humidity (30 to 50 percent RH) for 30 minutes or longer failed to recover when returned to high humidities. When

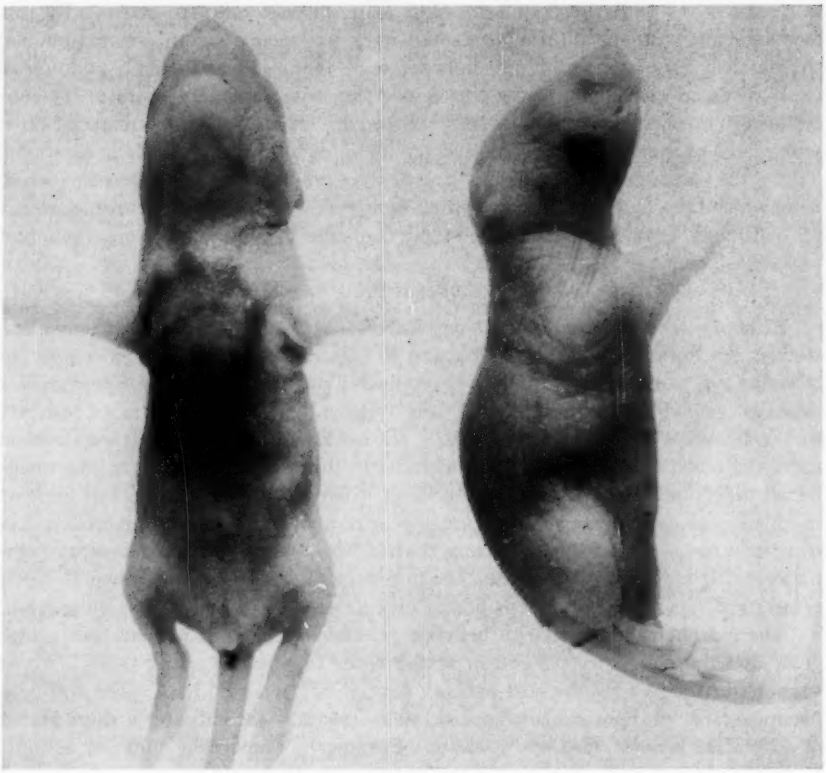

FIGURE 2. Suckling laboratory mice showing lesions produced by *H. liponyssoides* cultures. Hemorrhagic areas surround the punctures which are located in the right axillary region of the mouse on the left and on the cheek of the mouse on the right.

reared in tubes with unmoistened cotton plugs, over wet sand, or various salt solutions, few mites succeeded in reaching the deutonymphal stage. Moistening of one of the cotton plugs in each tube, to provide a gradient up to 100 percent relative humidity, reduced mortality rates to 4, 11, and 14 percent for the egg, larval, and protonymphal stages, respectively.

Deutonymphs and adults were more resistant to desiccation, but still required a very moist environment. Saturated salt solutions in sealed desiccating jars held at 20 C were used to provide 2 different humidities; daily opening of the jars caused some fluctuation in the indicated relative humidities. In each chamber, 30 deutonymphs and adults were kept in tubes and fed heparinized blood. Over dibasic potassium phosphate (92 percent RH) most mites died within 2 or 3 days, while over lead nitrate (98 percent RH) mortality was less than 10 percent during the first 10 days and some adult females lived over 3 months. *H. ambulans* kept over dibasic potassium phosphate reproduced regularly and all stages thrived.

Mass Culturing

Consistent success in rearing large numbers of mites on suckling laboratory mice

in plastic boxes with wet cotton and nest material was indicated above. By this technique and with subculturing at 2- to 4-week intervals, a colony was maintained continuously for over a year. The only servicing required was the daily addition of fresh mice and moistening of the cotton as necessary. Recovery of mites for subculturing or other purposes was accomplished by shaking the nest material on a wire screen over an enamel pan and picking up mites from the pan with an aspirator. Tilting the pan and tapping it lightly caused the eggs to collect in the lowest corner, from which they could be removed with a moistened brush. In subculturing, transfer of 10 to 20 gravid females to each fresh container at 3-week intervals was found most satisfactory.

DISCUSSION

Although their external morphology indicates a close relationship, *H. ambulans* studied by Furman (1959a, 1959b) and *H. liponyssoides* differ significantly in behavior and bionomics. *H. ambulans* produces living young, while *H. liponyssoides* deposits eggs which may take several days to hatch. *H. ambulans* is more resistant to low humidity than *H. liponyssoides*. *H. ambulans* will feed and reproduce on extracted blood and is not particular as to its freshness. This species can be maintained indefinitely on a diet of arthropods or dried blood, will feed on flea feces or cannibalize its own larvae and does not appear normally to pierce the unbroken skin of vertebrates. All evidence indicates that *H. liponyssoides* is an obligatory ectoparasite. It regularly pierces the skin to obtain the blood of experimental hosts, refuses arthropod food, and feeds poorly on extracted blood unless it is quite fresh.

The morphological similarity between *H. ambulans* and *H. liponyssoides* suggests that the obligatory parasitism of the latter is a recent acquisition. This is supported by aspects of the host-parasite relationship reported here. The formidable appearance of the wounds produced by *H. liponyssoides* indicates a short period of adaptation between host and parasite. Feeding by numbers of mites at a single wound suggests a recently acquired ability to penetrate the skin.

The results of experiments on feeding behavior indicate that odor is the chief stimulus involved. Storage of blood resulted in rapid changes, making it less attractive to the mites. Feeding was increased by conditions tending to improve the odor gradient emanating from extracted blood or a host. Thus, engorgement in screen-topped dishes was greater than in tubes where the odor of blood quickly filled the small space, and feeding on suckling mice increased when nest material was changed or ventilation improved.

SUMMARY

Haemogamasus liponyssoides has egg, larval, protonymphal, deutonymphal, and adult stages. Under optimum conditions in the laboratory, the cycle from egg to egg regularly is completed in less than 3 weeks. Only the deutonymphs, males and females normally feed or require food. They are obligatory parasites and pierce the skin of rodent hosts to feed on blood. The olfactory sense appears to be of primary importance in stimulation of feeding. The host-parasite relationship indicates a recent acquisition of the parasitic way of life. Detailed observations on the biology and development of all stages are presented, and a simple technique is described for mass culturing the mites on suckling laboratory mice.

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RESEARCH NOTE

METHOD FOR DETERMINATION OF THE NUMBER OF GYRODACTYLID TREMATODES PARASITIZING THE SKIN OF GOLDFISH.

Present techniques for the quantitative determination of the number of monogenetic flukes on the skin of a fish require the death of the host. An estimate of the worm burden of a live fish can be obtained by microscopical examination, but the accuracy of this method is questionable, especially when a large number of flukes is present. A procedure has been devised whereby a gyrodactylid (*Gyrodactylus elegans* var. A Mueller, 1936) that is a parasite on the skin of goldfish can be removed and counted without injury to the host. The chief advantages of this method are that a large number of flukes can be accurately counted, and the host can be used for further studies.

The apparatus for removal of the trematodes from the goldfish consists of a 50 ml conical centrifuge tube, ordinary plastic window screen and a 1 : 5500 solution of formalin. A 30-minute treatment with this dilution of formalin caused the flukes to detach before they were killed, and did not visibly damage the fish. Stronger solutions were unsatisfactory because they caused some damage to the fins of the fish, and because the flukes tended to die on and remain attached to the fish. Solutions weaker than 1 : 5500 either did not affect the trematodes or resulted in variable detachment within the 30-minute treatment period.

A 3-inch square of plastic screening is placed in the centrifuge tube in such a manner as to form an open sac at the top end of the tube. The excess screen then is cut away from the sac so that the screen lies flush against the sides of the tube. The bottom of the sac is positioned about 1 inch above the base of the centrifuge tube, and 40 ml of the 1 : 5500 formalin solution is added. The infected fish is placed in the tube and treated for 30 minutes, after which the screen containing the fish is removed with a pair of forceps, held over the mouth of the tube, and washed thoroughly with a fine stream of water from a squeeze bottle. The trematodes either are allowed to settle or are centrifuged and the supernatant fluid is removed and saved. The remaining portion then is transferred, with adequate washings, to a petri dish, and the number of flukes present is determined with the aid of a dissecting microscope. The supernatant fluid should be examined in the same manner because a few flukes occasionally may be trapped in the surface film. The treated fish also should be checked with the aid of a dissecting microscope in order to detect any flukes that were not removed by the treatment.

With the present technique it has been possible to obtain the complete isolation of *G. elegans* var. A from about 95 percent of over 100 goldfish tested. The intensity of infections in this series ranged from a few to several hundred trematodes per fish. In the few cases where this procedure was less than 100 percent effective, only 1 or 2 flukes out of an initial burden of several hundred remained on the host.

The above technique is simple, rapid and efficient, and it is conceivable that with modification it could be useful in studies on larger fish as well as on some other external fish parasites.

(This work is part of a study that is supported by Research Grant E-1619 from the Institute of Allergy and Infectious Diseases, National Institutes of Health. The authors wish to thank Dr. William Hargis, Virginia Fisheries Laboratory, for his aid in the identification of the trematode used in this study.)—JOHN C. PARKER AND A. JAMES HALEY, *Zoology Department, University of Maryland, College Park, Maryland.*

THE NASAL MITES (ACARINA) OF SOME WEST TEXAS FLYCATCHERS (TYRANNIDAE)*

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Nasal mites have been recovered from most, but not all, orders of birds. Considerable taxonomic work has been done on this group by Fain (1955, 1956, 1957), who has published a comprehensive review of the group through 1957 along with the descriptions of many new forms from Central Africa. Zumpt and Till (1955) have contributed much on this group from South Africa. In the Western Hemisphere, Strandtmann (1951), Strandtmann and Furman (1956), and Furman (1957) have contributed to the knowledge of this group in the United States. Castro (1948) and Pereira and Castro (1949) in South America have published on nasal mites from Brazil. They are also the only ones who have published on mites from the flycatcher group, but all of their work has been on the tropical forms of the family. A brief paper by Crossley (1951, unpublished thesis), on the internal anatomy of the pigeon nasal mites, is the only work as yet on the biology of this group of mites.

Bird nasal mites belong to three families. The family Rhinonyssidae (Vitzthum, 1935) of the suborder Mesostigmata is the most common group. The family Speleognathidae (Womersley, 1936) of the suborder Trombidiformes is also frequently found. The third family, Epidermoptidae (Trouessart, 1892), of the suborder Sarcoptiformes is relatively rare and has been reported from relatively few species of birds.

Measurements appearing in the descriptions are reported in microns.

This investigation concerns the nasal mites of flycatchers of the family Tyrannidae (Passeriformes) which frequent the South Plains of Texas. The birds were collected in the spring, summer, and fall of 1958 within a hundred mile radius of the city of Lubbock, Texas. Nasal mites were found in 7 of the 10 species collected.

For this study, a single family of birds was chosen to determine the amount of host specificity exhibited by these mites among a related group of hosts. The family Tyrannidae was selected because of the abundance of species on the South Plains and because the nasal mites of this particular family had not been investigated.

Mites were taken from the following flycatchers: Scissor-tailed flycatcher, *Muscivora forficata;* Say's Phoebe, *Sayornis sayus;* least flycatcher, *Empidonax minimus* olive-sided flycatcher, *Nuttallornis borealis:* western wood peewee, *Contopus sordidulus;* ash-throated flycatcher, *Myiarcus cinerascens;* and western kingbird, *Tyrannus verticalis.*

FAMILY RHINONYSSIDAE

Tyranninyssus n. g.

Medium sized mites (female body length 640 to 1150) with 2 or 3 dorsal shields of distinctive shape. Stigmata with short peritreme, dorsal and above coxa III. Sternal plate absent in

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female, but its location marked by nonstriated area. Remnants of sternal plate occasionally observed in males. Six sternal setae and 4 pores located on non-striated sternal area. Genital plate present, but no, or little, sclerotization, with median, longitudinal striations and pair of setae near posterior end; a pore located laterally, just off genital plate near each seta. Anal plate poorly defined and never sclerotized. roundish and terminated posteriorly in thickly pilose cribrum; 3 setae associated with anal opening. Movable segments of palp longer than fused coxae, tined tarsal seta absent; deutosternal teeth minute and very difficult to see in some species (*T. spinosus*), chelicerae rather stout, attenuated very slightly, if any; chelae rather prominent and form from one-fifth to one-tenth the total length of chelicerae. Four heavy, blunt setae are arranged in an oblique row on dorsum of genu III (not found on mites from hosts other than tyrant flycatchers).

The genus closely resembles *Paraneonyssus* but differs from it as follows: *Paraneonyssus* has 2 dorsal plates, *Tyranninyssus* may have 3; chelicerae much heavier and shorter in *Tyranninyssus* and chela much larger in proportion to chelicera than in *Paraneonyssus; Paraneonyssus* has a sternal plate and a tined palp seta; *Tyranninyssus* has neither. *Paraneonyssus* lacks the 4, aligned setae on genu III.

Generotype: *Tyranninyssus tyrannus* n. sp.

Tyranninyssus tyrannus n. sp.
(Pl. I, figs. 1–9)

An elongated mite, with 2 large and 2 small dorsal plates.

Female: (Pl. I, figs. 1–2, 4, 6–9) Body length (excluding gnathosoma), 670 to 830; average, 750. Width, 340 to 420; average, 370. Podosomal plate length, 260 to 270; average, 263. Width, 260 to 280; average, 270. Opisthosomal plate length, 320 to 340; average, 330. Width, 105 to 130; average, 126.

VENTER: (Fig. 9) Sternal area with 3 pairs of setae and 2 pairs of pores. Genital plate slightly longer than wide, with rounded posterior border and oval, membranous anterior margin; bearing 1 pair of setae near posterior border. A pore is associated with this plate laterally just off the edge near each seta. Anal plate oval, with 3 setae surrounding anal opening. The cribrum extends to dorsal side of opisthosoma. Opisthosoma with 5 pairs of setae and striations as illustrated.

Dorsum: (Fig. 1) Podosomal plate large, circular and with 7 pairs of setae. Two setae located near stigma, 1 anteriorly, 1 posteriorly and medially. Opisthosoma bears 1 elongated, large plate which has 2 small, somewhat triangular plates located laterally to its anterior border. The small plates support no setae, but 4 pairs are found on the large plate; large opisthosomal plate has sclerotized and slightly concave, slender median portion which gives illusion of 2 separate plates at first glance. Remainder of dorsum striated as illustrated and with 7 pairs of small, short setae.

Legs: (Figs. 1, 4, 8, 9) Moderately long and of medium thickness. Setae as illustrated, with the typical 4, blunt setae arranged obliquely on dorsum of genu III (figs. 1, 4). Tarsi II, III, and IV have 3 spur-like setae at ventral base of pretarsus, 2 projecting distally and 1 anteriorly. An area of sensory setae is found on the dorsal apex of tarsus I (fig. 8).

Gnathosoma: (Figs. 6, 7) About one-third the length of the idiosoma. Three pairs of setae on ventral surface and approximately 10 deutosternal teeth. Palps slender, with setae as illustrated (figs. 6, 7). Chelicerae taper very slightly from base to tip (fig. 2); chela about one-eighth the length of entire chelicera. Chelae poorly sclerotized and without teeth. Tectum membranous, with rounded tip; attached dorsally to sclerotized palpal base.

Male: (Pl. I, figs. 3, 5) Body length (excluding gnathosoma), 620 to 680; average, 650. Width, 300 to 330; average, 315. Podosomal plate length, 210 to 240; average, 230. Width, 210 to 250; average, 238. Opisthosomal plate length, 240 to 280; average, 258. Width, 110 to 140; average, 120. Very similar to female except for usual sexual differences. Pretarsus of leg I greatly shortened.

Immature forms: Nymphs similar to adults but have 2 long, pilose setae on dorsal opisthosomal apex (fig. 25), and lack genital openings. Larva unknown.

Host and Locality: Type host, *Nuttallornis borealis* (Swainson) the olive-sided flycatcher, collected in Lamb County, Texas, May 11, 1958. It contained 12 mites, 8 females, 4 males. Also 16 mites; 3 males, 1 nymph, and 12 females, collected in Lamb County, Texas, August 31 to September 11, 1958, from 2 *Contopus sordidulus* (Sclater), the western wood pewee.

Types: Holotype female, paratype female, male, and nymph are deposited in the United States National Museum, Washington, D. C. Remainder of specimens are in the acarology collection at Texas Technological College, Lubbock, Texas.

Remarks: Distinguishing features of this mite are 2 small opisthosomal plates and a nearly divided large opisthosomal plate.

Tyranninyssus tyrannisoides n. sp.
(Pl. II, figs. 10–17)

Similar to *T. tyrannus* but has 4 small, dorsal plates near anterior end of the large opisthosomal plate instead of 2; and opisthosomal plate more uniformly sclerotized for its entire length.

Female: (Pl. II, figs. 10–12, 14, 16–17) Body length (excluding gnathosoma), 730 to 1150; average, 904. Width, 350 to 490; average, 410. Podosomal plate length, 260 to 290; average, 270. Width, 250 to 280; average, 265. Opisthosomal plate length, 300 to 360; average, 320. Width, 110 to 130; average, 118.

Venter: (Fig. 17) Three pairs of sternal setae and 2 pairs of pores. Genital plate slightly longer than wide, ovate with a pair of setae near posterior end flanked laterally by a pore. Anal plate longer than wide, with indistinct margins. Three setae surround anal opening; paired setae longer than posterior one. Remainder of venter striated as illustrated and bearing 4 pairs of setae and 1 pair of pores.

Dorsum: (Fig. 10) Podosoma supports a large, round shield centrally which bears 7 pairs of setae and 2 pores. Opisthosoma has elongated plate down central axis that bears 4 pairs of setae. Two small plates flank either side of anterior end of this plate. The small plates support no setae but are well sclerotized. Remainder of dorsum striated and with 7 pairs of setae as illustrated.

Legs: (Figs. 4, 10, 11, 17) Of medium size with small, attenuated setae. Two, heavy, ventral, spur-like setae and an anterior, lateral one at bases of pretarsi. The distinctive, oblique row of setae present on dorsum of genu III (fig. 4). There is 1 elongate, attenuate seta on each trochanter ventrally. Tibiae 3 and 4 bear a long, attenuate seta dorsally.

Gnathosoma: (Figs. 12, 16) Deutosternal groove contains 10 teeth; palps slender with setae as illustrated; tined tarsal seta absent. Palps arise from heavily sclerotized base, which supports elongated tectum. Tectum membranous, its rounded tip extends to midway of palpal tibia. Chelicerae stout and slightly attenuated; chela one-tenth the length of chelicera. Chelae edentate and poorly sclerotized.

Male: (Pl. II, figs. 13, 15) Body length (excluding gnathosoma), 710 to 800; average, 760. Width, 360 to 380; average, 370. Podosomal plate length, 220 to 250; average 230. Width, 210 to 250; average, 235. Opisthosomal plate length, 270 to 280; average, 275. Width, 115 to 125; average, 120. Similar to female except for usual sexual dimorphism. Legs like those of female except pretarsus of leg I much shorter.

Immature forms: Larva small, with thick, short legs and no obvious sclerotization. Setae present, but vestigial. Chelicerae moderately thick and attenuated for a short distance just before the very small chelae.

Two nymphs were in the molting stage so that specific details of the anatomy were difficult to determine . They do have 2 long, pilose setae on the dorsal apex of the opisthosoma.

Hosts and locality: Type host, *Sayornis sayus* (Bonaparte), Say's Phoebe, collected in Lamb County, Texas, September 2, 1958. It contained 4 males, 2 nymphs, and 12 females. Two males, 1 larva, and 5 females were removed from 2 specimens of *Empidonax mimimus* (Baird and Baird) collected in Lamb County, Texas, August 31 to September 14, 1958.

Types: Holotype female, paratype male, female, and nymph are deposited in the United States National Museum, Washington, D. C. Remainder of the paratypes are in the acarology collection at Texas Technological College, Lubbock, Texas.

Remarks: Similar to *T. tyrannus* but has 5 opisthosomal plates instead of 3.

Tyranninyssus callinectoides n. sp.
(Pl. III, figs. 18–26)

The outstanding feature of this mite is the peculiar shape of the podosomal plate which resembles the body outline of the edible blue crab of the genus *Callinectes*.

Female: (Pl. III, figs. 18–20, 22, 24, 26) Body length (excluding gnathosoma), 640 to 790; average, 700. Width, 350 to 380; average, 360. Podosomal plate length, 240 to 250; average, 243. Width, 300 to 340; average, 320. Opisthosomal plate length, 260 to 290; average, 273. Width, 120 to 130; average, 126.

Venter: (Fig. 18) Three pairs of sternal setae and 2 pairs of pores. Genital plate small, oblong, with rounded ends, sclerotized center, and prominent striations; bearing 2 setae near posterior end, and a pore found just off the plate near each seta. Anal plate subterminal with the cribrum extending onto the dorsum; anterior border not clearly defined; with 3 setae, the 2 anterior being about twice as long as the posterior. Remainder of the venter striated as illustrated and with 4 pairs of setae and 2 pores.

Dorsum: (Fig. 26) The large podosomal plate is pointed laterally and rounded anteriorly

and posteriorly. Eight pairs of setae and 3 pairs of pores dispersed over surface of plate with occasional odd seta showing on some specimens. Opisthosomal plate elongated; its length twice its breadth. Four pairs of setae and 5 pairs of pores adorn the surface. Two small platelets located on each side of anterior end of opisthosomal plate; neither bear setae. Two setae are located between podosomal plate and stigma; just posterior to stigma is a pore. Remainder of dorsum with 5 pairs of setae and striations as illustrated.

Legs: (Figs. 18, 19, 26) Of medium build and rather long, 2 spurs at ventral base of pretarsi II, III, and IV, legs III and IV have several heavy, spur-like setae ventrally, but most of setae are short and attenuated except for an occasional elongated seta on tibia and tarsus.

Gnathosoma: (Figs. 20, 22, 24) Some areas highly sclerotized, especially dorsal base of tectum. Palps 5-segmented, with setae as illustrated (figs. 20, 22); 2 prominent sensory setae on tip of tibia. Tectum elongate, with a rounded terminus. Deutosternal groove contains seven teeth. Chelicerae slightly attenuated; chelae small and short with no teeth and not heavily sclerotized.

Male: (Pl. III, figs. 21, 23) Body length (excluding gnathosoma), 570; width, 310. Podosomal plate length, 220; width, 310. Opisthosomal plate length, 225; width, 110. Only 1 male specimen was available.

Dorsum and legs similar to female. Genital opening between coxae of 1st pair of legs. Posterior to genital pore, remnants of a sternal plate bearing 2 pairs of setae. Four, paired setae arising from a non-sclerotized, unstriated area between 2d and 3d pairs of legs. Remnants of a sclerotized ventral plate with a pair of setae and a pair of pores is located in the region of legs IV. Anal plate not quite so large as in female, but setal size and arrangement similar. Gnathosoma similar to that of female with exception of chelicera, which is equipped with well defined spermatodactyl (fig. 21).

Immature forms: One immature form was collected, a molting nymph. Similar to adult with exception of 2 long, pilose setae on posterior margin of opisthosomal plate (fig. 25), lack of genital opening, and smaller size. Length, 530; width, 280.

Host and locality: Described from *Myiarchus cinerascens* (Lawrence), the ash-throated flycatcher, collected in Briscoe County, Texas, April 26, 1958. One male, 1 nymph, and 3 females were removed from this bird.

Types: The holotype female, a paratype female, male, and nymph are deposited in the United States National Museum, Washington, D. C. Remaining paratype in the acarology collection at Texas Technological College, Lubbock, Texas.

Remarks: Differs from other species of the genus by the unique shape of the podosomal plate.

Tyranninyssus spinosus n. sp.
(Pl. IV, figs. 27–35)

An oval mite with 3 dorsal plates. The legs are thick and have heavy, blunt, spur-like setae ventrally.

Female: (Pl. IV, figs. 27–28, 31–35) Body length (excluding gnathosoma), 730 to 900; average, 810. Width, 430 to 540; average, 496. Podosomal plate length, 250 to 280; average, 265. Width, 240 to 350; average, 300. Opisthosomal plate length, 210 to 240; average, 225. Width, 170 to 290; average, 206. Pygidial plate length, 70 to 85; average, 81. Width, 70 to 105; average, 90.

Venter: (Fig. 35) With 6 paired sternal setae and 2 pairs of pores. Genital plate elongated, with rounded anterior margin, a somewhat truncate posterior margin, and bearing 2 setae on posterior half of plate; plate lightly sclerotized and bearing many longitudinal striations centrally. A pore flanking either margin in the setal area. Anal plate somewhat diamond-shaped with 3 setae surrounding anal opening; margins poorly defined, a thickly pilose cribum extends to dorsum of opisthosoma. Remainder of venter striated as illustrated and supporting 4 pairs of setae and 1 pair of pores.

Dorsum: (Fig. 27) Three plates; podosomal, opisthosomal, and pygidial. Podosomal plate elliptical, with truncated posterior margin and 6 pairs of setae and 2 pairs of pores. Four pairs of setae surround podosomal plate. Opisthosomal plate has 2 lateral, anterior expansions, 3 pairs of setae, and 1 pair of pores. The pygidial plate, located near tip of opisthosoma, is circular and has 1 pair of setae. Remainder of the opisthosoma striated as illustrated and with 5 pairs of setae.

Legs: (Figs. 27, 31, 34, 35) Legs stout with many heavy, blunt, spur-like setae, especially ventrally. Attenuate setae are located near tip of each tarsus; tarsus I with a group of sensory setae on dorsal apex (fig. 34). The distinctive setae on genu III not so perfectly aligned in this species as in others of this genus but are definitely of the same general arrangement (fig. 31). At ventral apex of tarsi II, III, and IV are 2 heavy, blunt setae that are similar in all the mites of this genus. Leg lengths are as follows: I, 570; II, 500; III, 520; IV, 640.

Gnathosoma: (Figs. 28, 29, 32, 33) The 3 pairs of setae are much longer and heavier than in most mites of this genus, especially the most anterior pair, which is quite large on this mite and vestigial in most species. Deutosternal teeth are cryptic and easily overlooked. Palps are 5-segmented with 2 clearly visible sensory setae on tibial apex and other smaller setae as illustrated. Dorsally, base of palps heavily sclerotized, but connected to idiosoma by a bulbous, non-sclerotized area (fig. 28). Elongated tectum extends to base of palp tibia and ends in an obtuse point.

Male: (Pl. IV, figs. 29, 30) Body length (excluding gnathosoma), 710 to 800; average, 750. Width, 440 to 460; average, 450. Podosomal plate length, 250 to 260; average, 255. Width, 290 to 370; average, 322. Opisthosomal plate length, 200 to 210; average, 205. Width, 190 to 200; average, 192. Pygidial plate length, 69 to 71; average, 70. Width, 80 to 90; average, 82. Length of the legs is as follows: I, 420; II, 400; III, 390; IV, 470.

Dorsally, identical to female. Ventrally the sternal plate is missing, but 6 sternal setae and 4 pores are arranged over the non-striated area. A small, sclerotized area in region of 4th pair of legs is a remnant of a ventral plate. A single seta is located laterally on either side of this plate. Legs almost identical to those of female with exception of pretarsus I, which is much shorter.

Immature forms: Unknown.

Host and Locality: The type host is *Muscivora forficata* (Gmelin), the scissor-tailed flycatcher, collected in Lubbock County, Texas, June 9, 1958. Other specimens of the same species were subsequently obtained from *Tyrannus verticalis* (Say), the western kingbird, collected in Crosby County, Texas, July 31, 1958; from *Tyrannus dominicensis* (Gmelin), the gray kingbird, collected in Puerto Rico by Mr. Ted Tibbets; and *Tyrannus tyrannus* (Linn.), the eastern kingbird, collected in Orient, Long Island, New York, 1948, by Roy Latham. A total of 32 mites was obtained: 3 from the gray kingbird; 5 from the western kingbird; and 24 from 4 specimens of the scissor-tailed flycatcher. Of the total, 6 were males, 27 were females; no immature forms were recovered.

Types: Holotype female is deposited, along with paratype male and female, in the United States National Museum, Washington, D. C. Other paratypes are in the acarology collection of Texas Technological College, Lubbock, Texas.

Remarks: Distinct from other species of this genus by the possession of 3 dorsal plates, large, heavy spines on the venter of the legs, and the cluster of 4 setae on the dorsum of genu III. These setae are peculiar to this group but are slightly misaligned in this species (fig. 31). The enlarged anterior setae on the venter of the gnathosoma are also an excellent distinguishing feature.

Genus *Sternostoma*

The genus *Sternostoma*, family Rhinonyssidae, was described by Berlese and Trouessart in 1889. Distinguishing features are: stigma without peritreme; mouth parts located ventrally; sternal plate present; anal plate usually terminal. The genus has recently been revised by Furman (1957).

Sternostoma tyrannus n. sp.
(Pl. V, figs. 36–41)

A thick, rather elongated mite with several short, blunt setae on the tarsi of the 3 posterior pairs of legs.

Female: (Pl. V, figs. 36–41) Length of idiosoma, 580 to 630; average, 612. Width, 320 to 340; average, 328. Podosomal plate length, 190 to 205; average, 196. Width, 210 to 220; average, 212. Opisthosomal plate length, 150 to 170; average, 159. Width, 120 to 140; average, 130.

Venter: (Fig. 36) Sternal plate oblong, well sclerotized, and with 3 pairs of setae. Genital plate elongate, constricted in middle, bearing a pair of lateral setae near rounded posterior end, well sclerotized, with a membranous anterior border overlying the transverse genital opening. Anal plate round in shape, with heavily sclerotized lateral and posterior margins (fig. 36). A pair of lateral setae present on plate at level of posterior edge of anal opening. Remainder of venter lightly striated, with 2 pairs of setae and 1 pair of pores.

Dorsum: (Fig. 41) Podosomal plate large, granular; obtusely pointed anteriorly; truncate posteriorly, lateral margins rounded; with 6 pairs of alveoli and 1 pair of setae on posterior, lateral margin. Stigma, without a peritreme, located over coxa IV. Opisthosomal plate reverse pear shaped, with 2 pairs of setae and 3 pairs of alveoli. A pore is located just off plate near posterior end. Remainder of dorsum lightly striated and supports 1 pair of setae near central, posterior apex of opisthosoma.

Legs: (Figs. 36, 38, 41) Legs I and IV are same length and longer (320) than legs II and III, which are of equal length (260). All leg segments, except tarsus, have very few and

very minute setae. Tarsus I has an area of sensory setae on dorsal apex (fig. 38), and 4 elongated, attenuate setae distally. Tarsi II, III, and IV have 2 long, attenuated setae and many short, heavy setae with blunt tips (figs. 36, 41). Claws visible on legs II, III, and IV, but are indistinct on leg I. Inner, lateral margin of coxa I has an ovoid, sclerotized area.

Gnathosoma: (Figs. 37, 39) Ventrally located with only part of palps visible dorsally (fig. 39). Two pairs of very small hypostomal setae; no deutosternal teeth. Palps have 4 movable segments; setae as illustrated. Tectum membranous, with rounded margin (fig. 39). Chelicerae attenuated, with a lateral, membranous protuberance in region of attenuation (fig. 40); chelae minute.

Male: Unknown.

Immature forms: Unknown.

Host and locality: Type host, *Nuttallornis borealis* (Swainson), the olive-sided flycatcher, collected in Lamb County, Texas, on September 2, 1958. Seven mites were removed from this bird, all females.

Types: Holotype female and 1 paratype deposited in the United States National Museum, Washington, D. C. Other paratypes are in the acarology collection at Texas Technological College, Lubbock, Texas.

Remarks: A pair of distinct, pointed setae on the posterior lateral angles of the podosomal plate; an anterior and a posterior pair of such setae on the opisthosomal plate, and a pair of similar, but somewhat larger setae on the dorsum near the posterior end, clearly distinguish this mite from others of the genus.

Family Speleognathidae

Genus *Boydaia*

The genus *Boydaia* was established by Womersley (1953) to take in a new mite from an Australian toad. The genus is characterized by 3-segmented palp and absence of eyes and scutum.

Boydaia tyrannis Ford
(Pl. VI, figs. 42–48)

Ford, Hedwig Geiger 1959: 380.

Similar to *B. clavata* Fain (1955), with the exception of the heavy sclerotization at the base of the gnathosoma and the forked empodium of tarsus I. No satisfactory method has been found for distinguishing the sex of these mites, so they will be referred to here as adults and immatures.

Adults: (Pl. VI, figs. 43–47) Length of idiosoma, 360 to 530; average, 460. Width, 260 to 430; average, 356. In life these mites are a pale yellow in color, with a midlongitudinal stripe of white.

Venter: (Fig. 45) Two poorly defined plates present, genital and anal. Anal plate quite small, located at posterior extremity, and devoid of setae. Immediately anterior to the anal plate are the 2 lateral halves of the genital plate, each bearing 3 plumose setae (fig. 45). These plates are not nearly so prominent as we have illustrated. There are 3 pairs of midventral setae in the podosomal area; 2 pairs between coxae IV and the genital area, and 2 pairs between genital and anal pores. Occasionally there are 2 or more setae in the genital area. The illustration shows 4 extra setae, which is not unusual.

Dorsum: (Fig. 47) Relative size and arrangement of setae about as illustrated. Each pseudostigmatic organ has just anterior to it a very small, pilose seta which our illustration does not show.

Legs: (Figs. 43, 45, 47) Relatively slender, with internal, sclerotic bars. All setae are pilose except 1 nude sensory seta mid-dorsally on tarsus I. All leg setae are more slender than drawing indicates. Coxae I, II, and IV have 1 seta each; coxa III has 2 setae, 1 midventral and 1 on anterior-apical margin. Tarsal claws unmodified, empodium Y-shaped and pilose (fig. 43).

Gnathosoma: (Fig. 44) Well sclerotized reticulations at ventral base, with 2 pairs of short, pilose setae just anterior to the reticulations. Palps apparently 3-segmented, with 3 pilose and 1 nude setae on apical segment. Chelicerae rather short, broadly cone-shaped, unfused. Chelae very slender, needle-like, about one-fourth as long as chelicera. They arise ventrally near apex of chelicera and bend sharply forward.

Immature forms: (Fig. 42, 46, 48) One nymph was observed. It appeared to be identical to adult except for absence of genital opening. Nine larvae were recovered (figs. 42, 46, 48). Measurements: length, 270 to 350; average, 320. Width, 220 to 260; average, 240. Similar to adult but with only 3 pairs of legs; pseudostigmatic organs more elongated and not so broad distally as in adult; legs thicker in proportion to their length than in adult; fewer setae both dorsally and ventrally; tarsus I with vestigal empodium (fig. 42).

Host and locality: Tyrannus verticalis (Say), the western kingbird, collected in Garza, Baily, and Lamb Counties, Texas, June to August, 1958. A total of 88 mites were recovered from 11 birds. No other species of mite was found in association with these mites.

Remarks: This mite is distinguished from other members of the genus by the forked empodium on tarsus I and by the sclerotized striations on the venter of the gnathosoma. The spatulate pseudostigmatic organs are peculiar to this species and to *B. clavata* Fain.

Ford (1959) described the species from the eastern kingbird, *Tyrannus tyrannus* (Linnaeus). We can find no significant differences between our specimens from the western kingbird and Ford's descriptions and illustrations. Miss Ford mentioned a "peculiar sac-like structure present in dorsal aspect of tibia I." This we were unable to find.

COLLECTING DATA

A. *Nuttallornis borealis* (Swainson), Olive-Sided Flycatcher

Two specimens were collected in Lamb County, Texas, on May 11, and September 2, 1958. Both birds were parasitized: the first, by twelve specimens of *Tyranninyssus tyrannus,* 8 females and 4 males; and the second, by 7 specimens of *Sternostoma tyrannus,* all females. The 2 birds were the only members of this species observed in the collecting area during 1958.

B. *Contopus sordidulus* (Sclater), Western Wood Pewee

Seven birds were collected in Lamb County, Texas, during the period August 31 to September 11, 1958. Two contained nasal mites; 1 had 7 specimens, a male and 6 females; the other had 9 mites, 1 nymph, 2 males, and 6 females. Mites were *Tyranninyssus tyrannus.*

C. *Sayornis sayus* (Bonaparte), Say's Phoebe

Two birds collected: 1 in Lamb County, Texas, September 2, 1958, which contained 18 specimens of *Tyranninyssus tyrannisoides,* 2 nymphs, 4 males and 12 females; the other in Lubbock County, Texas, September 20, 1958, which contained no mites.

D. *Empidonax minimus* (Baird and Baird), Least Flycatcher

Ten birds collected from August 31 to September 14, 1958, in Lamb County, Texas. Two birds, contained *Tyranninyssus tyrannisoides.* One had 6 mites, 1 larva, 2 males, and 3 females; the other had 2 female mites.

E. *Myiarchus cinerascens* (Lawrence), Ash-Throated Flycatcher

Two specimens collected. One in Briscoe County, Texas, April 26, 1958, contained 5 *Tyranninyssus callinectoides,* 1 nymph, 1 male, and 3 females; the other taken in Garza County, Texas, June 10, 1958, had no mites.

F. *Muscivora forficata* (Gmelin), Scissor-Tailed Flycatcher

Ten birds were collected, in the West Texas Counties of Garza, Lubbock, and Bailey in June and July, 1958. Four birds were parasitized by *Tyranninyssus spinosus;* 28 mites: 5 males, 19 females were recovered.

G. *Tyrannus verticalis* (Say), Western Kingbird

Thirty-three specimens were collected in Garza, Cochran, Bailey, Lamb, and Crosby Counties during June, July, and August of 1958. Eleven birds were infected with *Boydaia tyrannis* Ford. A total of 88 mites; 79 adults, and 9 larvae were recovered. One bird, collected in Crosby County, Texas, contained 1 male and 4 female specimens of *Tyranninyssus spinosus;* this was the only species of mites taken from this bird.

DISCUSSION

The rhinonyssid family of mites are slow and sluggish of movement. This fact, coupled with their inability to survive more than a few minutes when exposed to desiccating effects of the atmosphere, is probably the greatest contributing factor to the extreme degree of host specificity which they display. A different genus of family Rhinonyssidae is found in each order of our modern birds with exception of order Passeriformes, which is parasitized by several genera.

It is not known exactly when or how mites of the family Rhinonyssidae are transmitted from one host to the other. It would seem obvious, however, that transfer must occur at some time when the mite will not have to be outside the nasal passage for more than a few moments. Such a time could occur when a parent bird is depositing food in the mouth of its young, or during some of the courtship displays of affection when the bills of two birds are intimately associated. It would be almost an impossibility, with these restricting factors, for a passeriform bird, such as a sparrow, to transmitt its nasal mites to an anseriform bird such as a duck, because of the complete non-association of the two birds.

Among the various species of flycatchers the incidence of parasitism averaged 30 percent. The relatively low incidence indicates that transmission from one host to the other is not very efficient.

It has long been possible to know the order of the host of a particular rhinonyssid mite by the genus to which the mite belongs; now in many cases we can also identify the family of the host by its mite. Mites from various members of the swallow family, Hirundinidae, for example, although being typical of genus *Ptilonyssus,* have body setae that are heavy and blunt and are in this respect unlike *Ptilonyssus* species from other families of Passeriformes. Mites from the flycatcher family Tyrannidae have 4 heavy setae aligned on the dorsum of genu III. This setal cluster is found in no other group of bird mites, so that a mite having this characteristic is immediately identified as having a tyrannid host. It is possible, and quite probable, when additional information is available, that the host specificity displayed by this family of mites will be an asset to the ornithological taxonomist in arriving at a better understanding of the relationships of the various families of birds.

SUMMARY

Ten species of tyrant flycatchers (Tyrannidae: Passeriformes), were examined for nasal mites (Acarina). The birds were taken in West Texas during the months of June to September, 1958. The mites that were recovered and the host in which they were found are: (1) *Tyranninysus tyrannus* n. g., n. sp. (generotype) from the olive-sided flycatcher, *Nuttallornis borealis* (type host) and the western wood peewee, *Contopus sordidulus;* (2) *Tyranninyssus tyrannisoides* n. sp. from Say's phoebe, *Sayornis sayus* (type host) and the least flycatcher, *Empidonax minimus;* (3) *Tyranninyssus callinectoides* n. sp. from the ash-throated flycatcher, *Myiarchus cinerascens;* (4) *Tyranninyssus spinosus* n. sp. from the scissor-tailed flycatcher, *Muscivora forficata* (type host) and from the western kingbird, *Tyrannus verticalis,* the gray kingbird, *Tyrannus dominicensis,* and the eastern kingbird, *Tyrannus tyrannus;* (5) *Sternostoma tyrannus* n. sp. from the olive-sided flycatcher, *Nuttallornis borealis;* (6) *Boydaia tyrannis* Ford from the western kingbird, *Tyrannus verticalis.*

A unique feature of the new genus is an oblique row of four setae on the dorsum of genu III.

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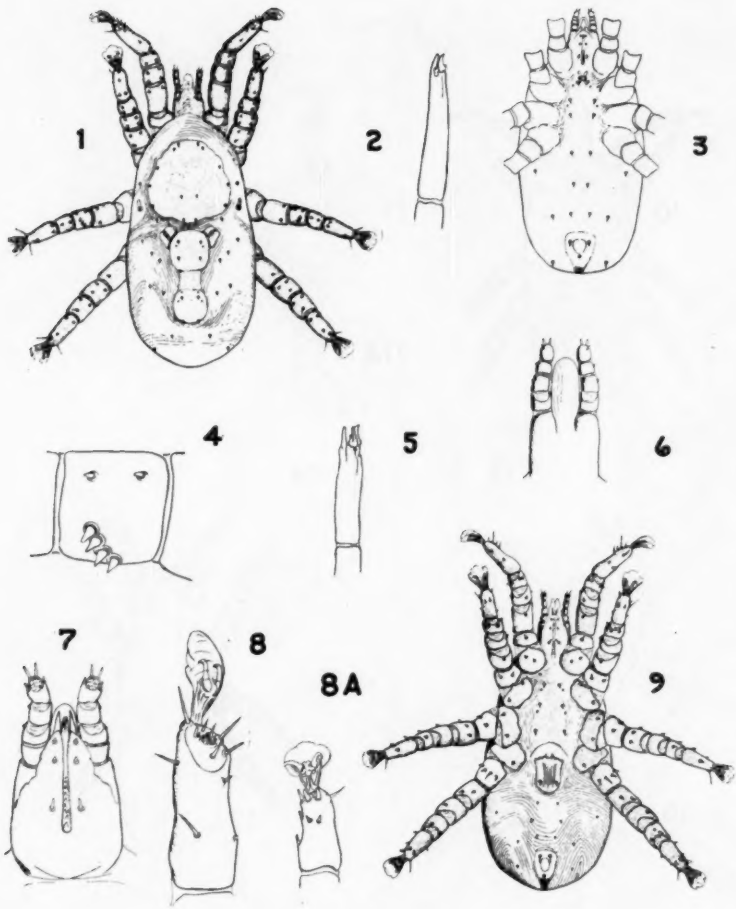
ANNOUNCEMENT

All members of the Society who have served at any time as parasitologists in the U. S. Army are requested to contact the following without delay:

LT COL LYMAN P. FRICK, MSC,
Sixth US Army Medical Laboratory,
Fort Baker, California.

A history of the U. S. Army Medical Service Corps is now in preparation. Since this will also involve all corps that anteceded the present one, the story of the old Sanitary Corps, Army of the United States, will constitute a major part of the history. Information is urgently required on the duties, assignments, accomplishments, etc., of all former Army parasitologists.

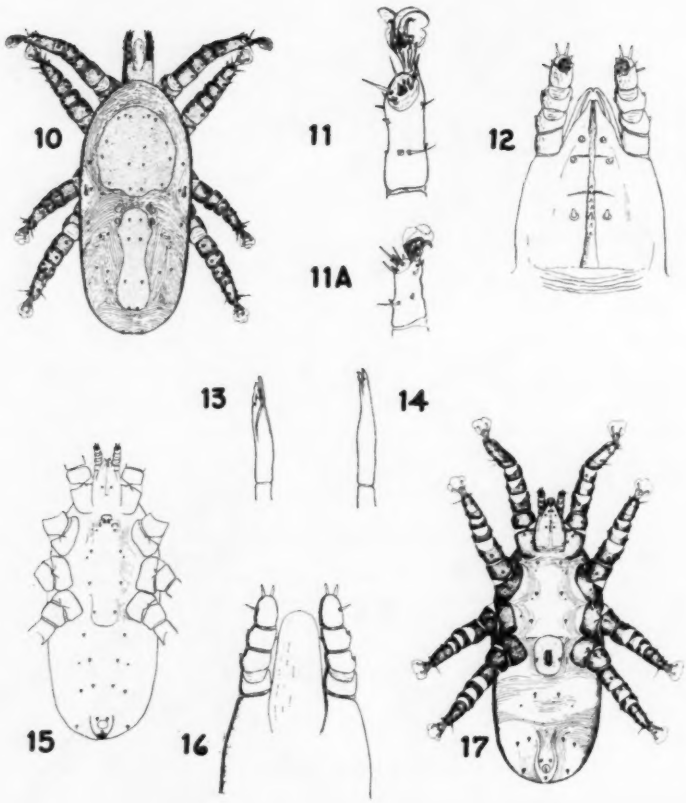
PLATE I



EXPLANATION OF PLATE I

Tyranninyssus tyrannus: 1, dorsal view of female; 2, female chelicera; 3, ventral view of male; 4, genu III, dorsal view; 5, male chelicera; 6, gnathosoma of female, dorsal view; 7, gnathosoma of female, ventral view; 8, tarsus I of female, dorsal view; 8a, tarsus II of female, ventral view; 9, ventral view of female.

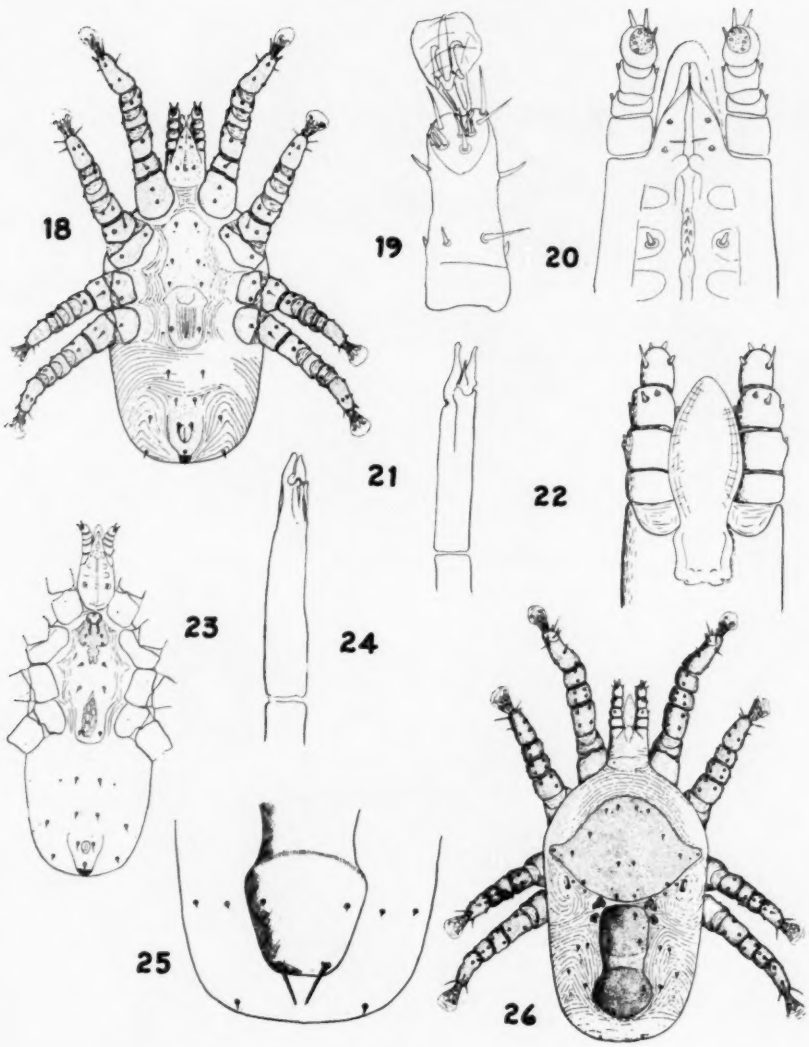
PLATE II



EXPLANATION OF PLATE II

Tyranninyssus tyrannisoides: 10, dorsal view of female; 11, tarsus I of female; 11a, tarsus I of male; 12, gnathosoma of female, ventral view; 13, male chelicera; 14, female chelicera; 15, male, ventral view; 16, gnathosoma of female, dorsal view; 17, ventral view of female.

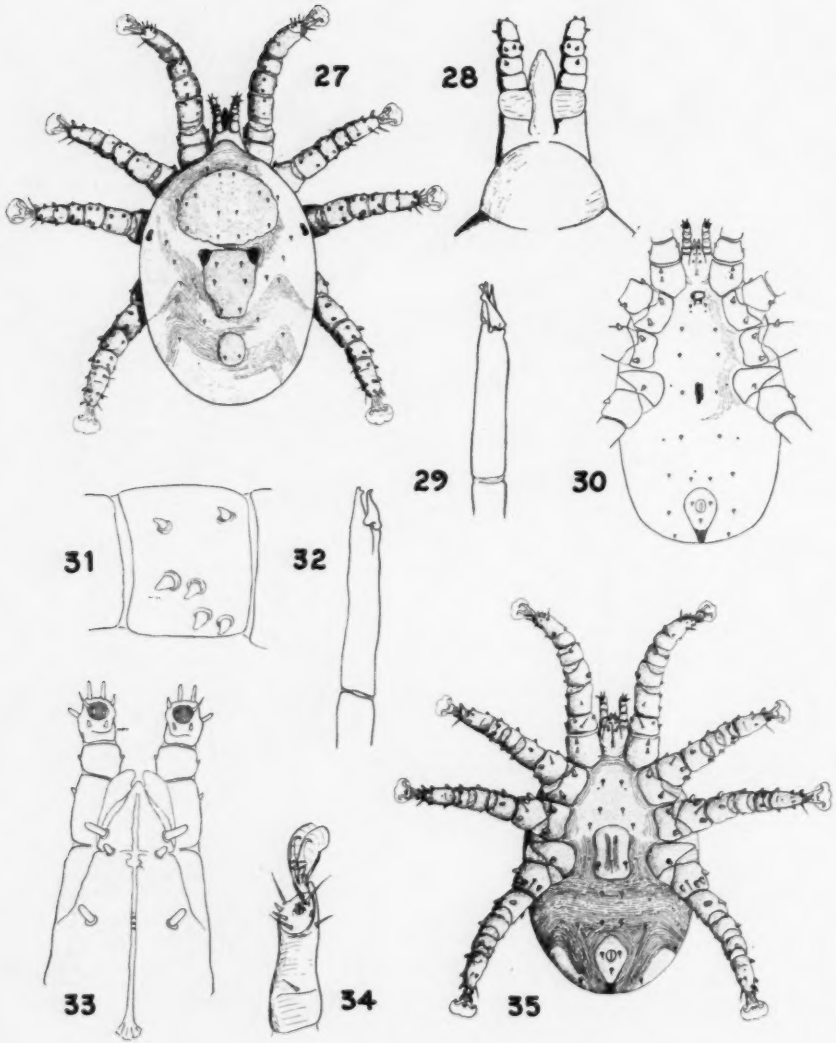
PLATE III



EXPLANATION OF PLATE III

Tyranninyssus callinectoides: 18, ventral view of female; 19, dorsal view of tarsus I, female; 20, gnathosoma of female, ventral view; 21, male chelicera; 22, gnathosoma of female, dorsal view; 23, ventral view of male; 24, female chelicera; 25, opisthosoma of nymph, dorsal view; 26, dorsal view of female.

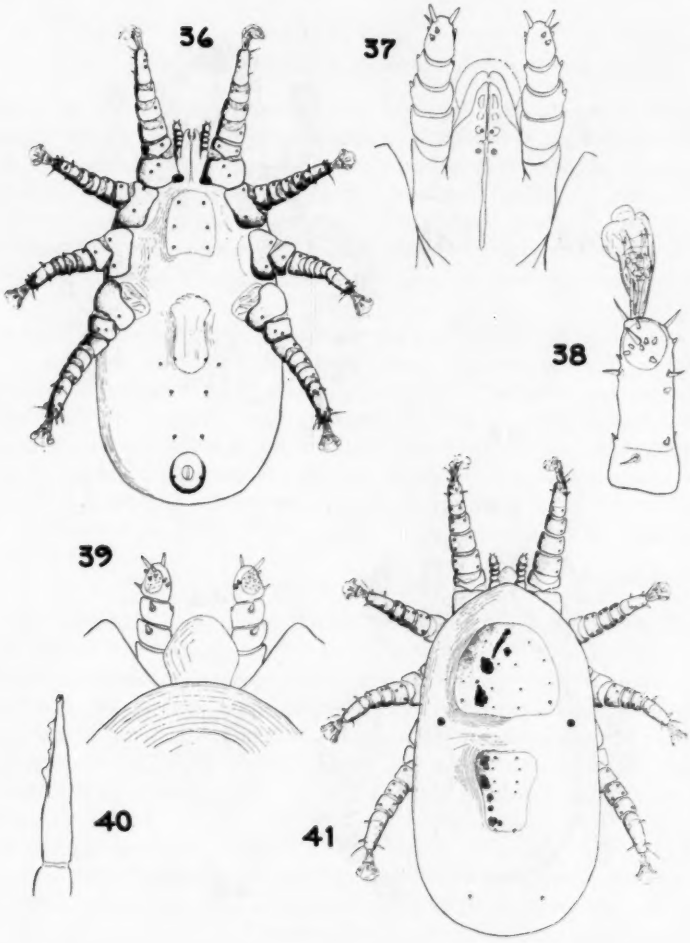
PLATE IV



EXPLANATION OF PLATE IV

Tyranninyssus spinosus: 27, dorsal view of female; 28, gnathosoma of female, dorsal view; 29, male chelicera; 30, ventral view of male; 31, genu III of female, dorsal view; 32, female chelicera; 33, gnathosoma of female, ventral view; 34, tarsus I of female; 35, ventral view of female.

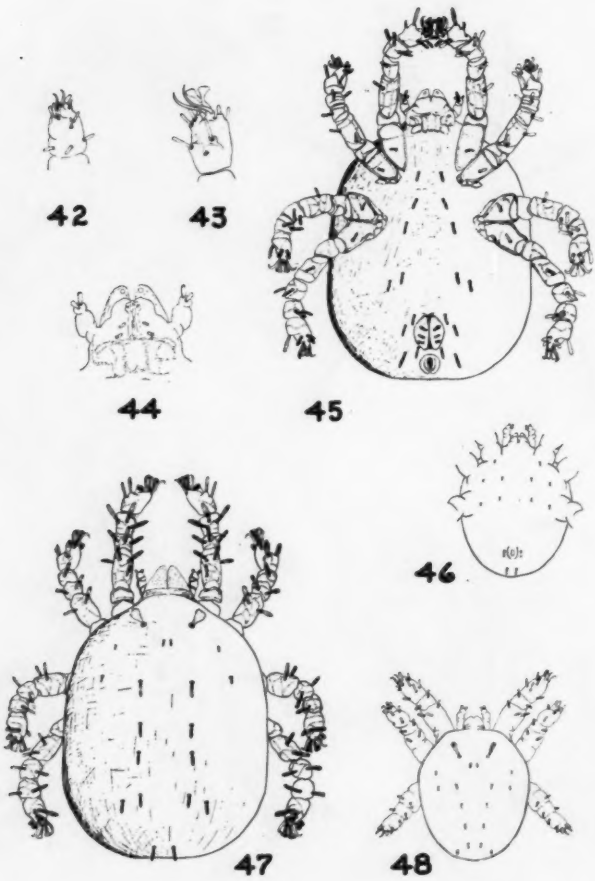
PLATE V



EXPLANATION OF PLATE V

Sternostoma tyrannus: 36, ventral view of female; 37, gnathosoma of female, ventral view; 38, tarsus I of female, dorsal view; 39, gnathosoma of female, dorsal view; 40, chelicera of female; 41, dorsal view of female.

PLATE VI



EXPLANATION OF PLATE VI

Boydaia tyrannis: 42, tarsus I, larva; 43, tarsus I, adult; 44, gnathosoma of adult, ventral view; 45, ventral view of adult; 46, ventral view of larva; 47, dorsal view of adult; 48, dorsal view of larva.

A NEW SPECIES OF *PIMELIAPHILUS* (ACARINA: PTERYGOSOMIDAE) PARASITIC ON SCORPIONS, WITH DISCUSSION OF ITS POSTEMBRYONIC DEVELOPMENT*

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Mites of the family Pterygosomidae have attracted much attention because of their parasitic habits. The majority of species now known are external parasites of lizards, but one genus contains species that are parasites of arthropods. The unique structural modifications of pterygosomid mites, developed to meet the needs of their unusual parasitic existence on various lizard hosts, have been discussed in some detail by Lawrence (1935, 1936), and some studies of insect parasitism by *Pimeliaphilus podapolipophagus* Tragardh and its biology have been reported by Cunliffe (1952).

In my experience the trombidiform mites most frequently encountered as ectoparasites of scorpions in the field have been members of the family Erythraeidae. Mites of this family, in their larval stages, are commonly parasitic on many arthropods. The discovery of pterygosomid parasites of Mexican scorpions was startling and the collections made following this discovery presented an excellent opportunity to investigate the developmental cycle of this species. Since all collections of the hosts, with their attached parasites, were placed directly in preservative in the field, the following discussions of the developmental biology of the parasitic mite is of a presumptive nature.

SYSTEMATIC POSITION

There are currently 8 genera in the family Pterygosomidae. Seven of these genera are represented only by species that are parasitic on lizards. The genus *Pimeliaphilus,* previous to the recognition of the new species described in this paper, included *P. podapolipophagus* Tragardh, a parasite of beetles and cockroaches; *P. triatomae* Cunliffe, a parasite of triatomid bugs, and *P. isometri* Cunliffe (1949), a parasite of scorpions in the Philippine Islands. The generic diagnosis of *Pimeliaphilus,* to which the new species described in this paper is added, will require slight modifications in order to accommodate this new mite. (Ocular plate with 1 seta, present in previous species, is absent in the new species. The new species has fewer than 13 pairs of dorsal setae, the characteristic number for the genus being 13 pairs.) It is apparent, however, that these modifications should be made, for the new species obviously belongs in this group. Since the genus was known from only 3 previously described species, such alterations of the generic diagnosis are to be expected.

Genus *Pimeliaphilus* Tragardh

Pimeliaphilus Tragardh, 1904, Acariden aus Agypten und dem Sudan, 1. Results of the Swedish Zoological Expedition to Egypt and the White Nile 1901, 20: 31-46; Cunliffe, 1952, Proc.

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Ent. Soc. Wash. **54:** 159–160.

Type of genus: *Pimeliaphilus podapolipophagus* Tragardh, monobasic and by original designation.

Palpus 5- or 6-segmented, with tarsus greatly reduced; legs arranged in 2 distinct groups, 2 front pairs directed anteriorly, hind pairs directed posteriorly; propodosomal shield always distinct in female; with 11 to 13 pairs of dorsal setae (including humerals) arranged in transverse rows or irregularly situated; body longer than wide; integument conspicuously striated over most of body; 1 pair of eyes which may or may not be borne on small plate with an associated seta; all legs with 2 claws, each claw bearing 2 prominent tenent hairs; whiplike sensory setae arising from specialized pits on tibiae I, III, and IV; tarsus I with a single pair of duplex setae, the proximal member minute.

Pimeliaphilus rapax n. sp.

Female (Fig. 8): Length, from tips of palpi to apex of hysterosoma, 770 microns; greatest width, 580 microns. Gnathosoma large and distinct; palpi five-segmented, with bases (coxae) fused; hypostome prominent; partially enveloping chelicerae. Palpal tibia with 2 stout, clawlike setae, 1 terminal and 1 subterminal on segment, and 2 unmodified setae. Palpal tarsus greatly reduced, articulating on ventral subterminal surface of tibia, with 1 peglike and 4 normal setae. Palpal coxa with 1 seta; palpal femur and genu each with 2 setae. Chelicera with dorsal fixed digit (telopodite) elongate, knobbed apically and its tip armed with a spine; movable ventral digit (endite) bladelike, its dorsal edge fitting into longitudinal ventral groove of dorsal digit, and its ventral edge fitting into a similar groove in the hypostome. The hypostome heavily sclerotized especially at its apex, which is curved to embrace both apical elements of the chelicerae. Dorsal propodosomal shield quadrangular, with a pair of setae at anterior fifth of shield and 1 seta at each of the posterolateral extremities of shield. Nine pairs of dorsal setae excluding the 2 pairs on shield. One pair of lateral eyes located dorsally in region of coxae III. Dorsal integument finely striate except for propodosomal shield. Ventral integument with fine striae except on coxal and anal plates. Four pairs of ventral setae excluding the 2 pairs on each coxae plate and the 3 pairs on anal plate. All legs with 6 segments. Coxae II and III, on each side, fused to form coxal plate; coxae III and IV similarly fused; each of the 4 coxal plates with a pair of setae. Duplex setae of tarsus I with proximal member minute. Tibiae I, III, and IV each with a very long seta; tarsus II with one specialized lanceolate seta. All claws greatly reduced, knoblike, each with 2 conspicuous tenent hairs; empodia minute, apically bilobed.

Male (Fig. 6): Length, from tips of palpi to apex of opisthosoma, 347 microns, greatest width, 270 microns. General appearance strikingly different from female; legs much longer and more slender and gnathosoma much smaller. Dorsal propodosomal plate absent. Dorsal striae indistinct in mid-dorsal region of body. Genital opening dorsal and subterminal, situated at apex of greatly elevated and pointed hump near tip of opisthosoma (conspicuous in unmounted specimens). Eleven pairs of dorsal setae, excluding single pair of anals. One pair of ventral setae, excluding setae of coxal plates. Aedeagus large, elongate and conspicuous.

Holotype: Gravid female, off scorpion (*Vejovis punctatus punctatus*) collected at Monte Alban ruins near Oaxaca, Mexico, August 15, 1955, by R. E. Beer.

Allotype: Male, same data as holotype.

Location of types: Holotype, allotype, and series of paratypes in the Snow Entomological Museum, The University of Kansas. Paratypes also deposited in the United States National Museum.

PRE-ADULT STAGES

Egg: Many eggs were recovered from the hosts examined. These were attached to the scorpion's body singly or in a cluster, usually the latter. The largest cluster consisted of 12 eggs and this cluster was covered and interspersed with loosely woven fibrous strands, suggesting the "tent" canopy described by Goodwin (1954) as characteristic for *Geckobiella texana.* Single eggs and small clusters often had no fibrous strands associated with them, but were simply glued to the body surface of the host. In view of the large numbers of mites in all stages of development recovered from many host individuals, and repeated association of the larger egg masses with shriveled carcasses of females, it seems apparent that eggs are frequently, if not characteristically, deposited while the female remains fixed in her original feeding position on the host.

Larva (Fig. 1): The present author observed no indications of the existence of a *"deutovum"* stage in this species. However, the possibility that such a stage exists does remain. Larvae are apparently very active, many of them washing free from the host immediately following immersion of the latter in preservative. The larval stage terminates while its mouthparts are still inserted in the host. The nymphochrysalis develops within the larval skin and the

latter apparently ruptures when the nymphochrysalis is completely formed. The rupture is ordinarily a tearing of the larval ventral integument at the base of the gnathosoma.

Nymphochrysalis: Obviously a quiescent stage, this form invariably is surrounded by remnants of the larval skin. Indeed, it is this mechanism that apparently prevents the nymphochrysalis from dropping from the host, for larval mouthparts remain inserted in host tissues. The nymphochrysalis (fig. 7) has 4 pairs of appendages tapering to bluntly rounded tips. The integument is conspicuously striate, except at tips of legs and the entire gnathosoma. The latter lacks detailed differentiation of components seen in active stages. Setae are absent. Dorsally, near the posterior end of the body is a conspicuous plate bearing 2 stout, caudally-directed, pointed processes. These invariably puncture the old larval skin, and hence may serve the purpose of adding substantially to the anchorage of the nymphochrysalis on the host.

Nymph (Fig. 3): The nymph figured was still embraced by pieces of larval and nymphochrysalis integument. Hence it is undoubtedly a very young nymphal specimen. It resembles the female in most details of its morphology, the most notable exceptions being its hexagonal propodosomal shield that envelops only 1 pair of the dorsal setae, and the presence of 1 dorsolateral and 1 ventrocaudal pair of pores on the opisthosomal integument. Because of the nearly identical morphology of the mouthpart and leg appendages of nymphs and females, the figures of these structures (figs. 2, 4, and 5) in the nymph (for which better material was available), rather than the female, are presented.

Imagochrysalis: This stage is very similar to the nymphochrysalis. Like the latter it depends upon the inserted mouthparts of the nymph and the caudal spines for anchorage on the host. The imagochrysalis terminates with the emergence of the adult. In some cases emergence does not occur, in females, but the same feeding site used by the nymph is used by the adult. This results in a female with remnants of nymphal and imagochrysalis integuments partially shrouding her body and remnants of the nymphal mouthparts inserted into the host adjacent to her feeding puncture. This apparently does not occur often and it is probable that adults move about actively before settling down to feed. Like *Geckobiella texana,* the male probably passes directly from the larval to the adult stage with the possible intervention of an imagochrysalis between the two. No evidence was found to indicate the route of male development, however.

General remarks: More than 400 mites were recovered from 16 specimens of *Vejovis punctatus punctatus* collected at the type locality. Only 5 specimens in this large collection were males. None of the males was attached to the host in a feeding position. This suggests that males are rare and that they possibly are not parasitic. Further evidence of their non-parasitic nature is seen in the greatly reduced gnathosoma which may make feeding impossible. Two species of scorpions, *Vejovis punctatus punctatus* Karsch and *Centruroides thorelli* Kraepelin were exceedingly abundant in the Monte Alban ruins area. Several of each often occurred under a single rock. The pterygosomid parasite, however, was not found on any of the many specimens of *Centruroides* examined. The extent of parasitism of *Vejovis* was quite variable from specimen to specimen. Some individuals were not infested with mites, others had large numbers of the parasites. Preferred attachment sites appear to be the conjunctivae where pedipalps and legs join the cephalothorax and similar membranous areas separating the tail (abdominal) segments. Some specimens were attached to other membranous areas of the body, however.

During the feeding operation by all of the parasitic stages, a mucillaginous secretion is produced in the oral region. This flows out over the integument of the host to form an adhesive disc when it dries. The secretion thus could provide a substantial anterior anchor for the mite, which probably facilitates penetration of host tissues by the extensible elements (endites) of the chelicerae. It is also probable that the spinelike processes at the apices of the cheliceral telopodites also serve a useful function in stabilizing the anchorage.

The 4 species in the genus *Pimeliaphilus* may be separated easily with the following key to the females:

1. Dorsal propodosomal shield rectangular 2
   Dorsal propodosomal shield triangular or pentagonal 3
2. With 13 pairs of dorsal setae, 3 pairs situated on propodosomal shield; palpal genu and femur united ..... *podapolipophagus* Tragardh
   With 11 pairs of dorsal setae, 2 pairs on propodosomal shield; palpal genu and femur distinct ..... *rapax,* n. sp.
3. Shield pentagonal; palpal genu and femur coalesced ..... *isometri* Cunliffe
   Shield triangular; palpal genu and femur distinct ..... *triatomae* Cunliffe

SUMMARY

A new species of pterygosomid mite, *Pimeliaphilus rapax,* parasitic on the scorpion *Vejovis punctatus punctatus* Karsch is described. Females ordinarily lay eggs

in clusters on the host which, upon hatching, produce larvae that probably feed on scorpion blood. Larvae destined to develop into females pass through a quiescent nymphochrysalis stage followed by a parasitic nymphal stage. The nymph then transforms into the quiescent, non-feeding imagochrysalis which subsequently gives rise to the adult female. Males are apparently quite active and evidence supports a claim that, as adults, they may not be parasitic. It is also possible that larvae transform directly into adult males, rather than passing through the several intervening stages of female development.

There is some evidence of host specificity and predilection for feeding sites in this new parasitic mite species. The functional anatomy of the feeding mechanism of the mite is also discussed in some detail.

ACKNOWLEDGMENT

The author takes this opportunity to thank Dr. Herbert L. Stahnke of Arizona State University for identification of the scorpion species encountered in this study.

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EXPLANATION OF PLATES

PLATE I

FIGURE 1. Larva of *Pimeliaphilus rapax*, new species, dorsal and partial ventral aspects.
FIGURE 2. Chelicera of *P. rapax*, dorsal aspect with apex slightly rotated. cb, cheliceral base (coxa); da, dorsal arm (telopodite); pr, peritreme; te, tectum; va, ventral arm (endite). Drawn from nymph.

PLATE II

FIGURE 3. Young nymph of *P. rapax*, dorsal and partial ventral aspects.
FIGURE 4. Tarsus I of young nymph, *P. rapax*.

PLATE III

FIGURE 5. Apex of palpus of young nymph, *P. rapax*.
FIGURE 6. Adult male of *P. rapax*, dorsal and partial ventral aspects.

PLATE IV

FIGURE 7. Nymphochysalis of *P. rapax*, ventral aspect.
FIGURE 8. Adult female *P. rapax*, ventral view of anterior portion of body. Drawn from holotype.

PLATE I

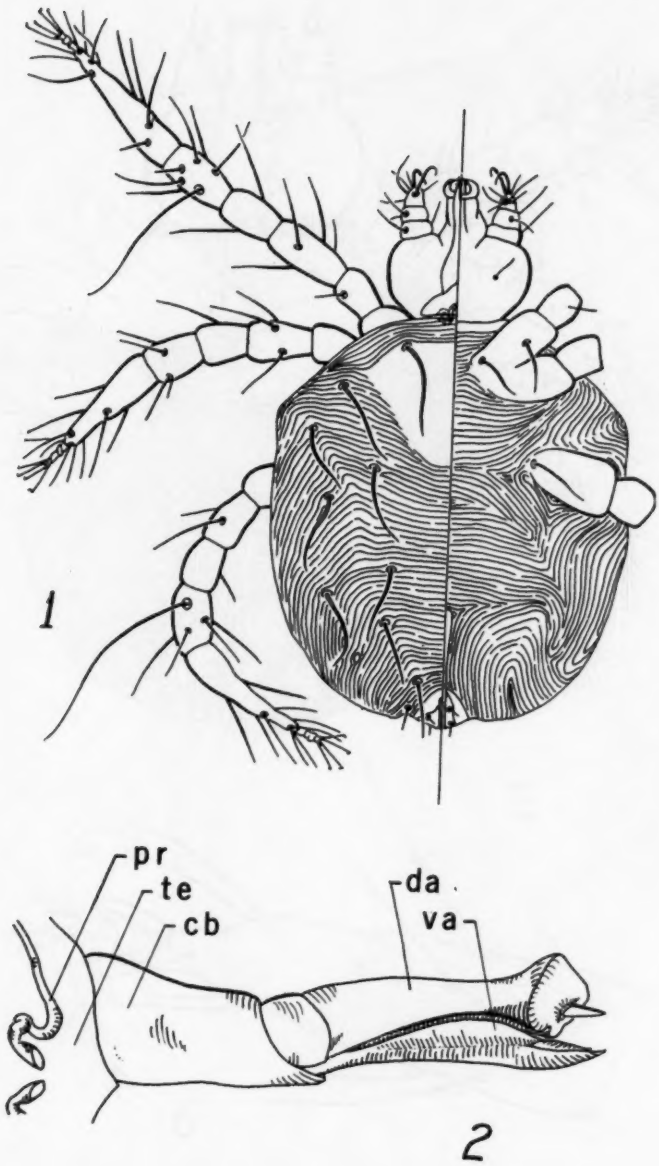


PLATE II

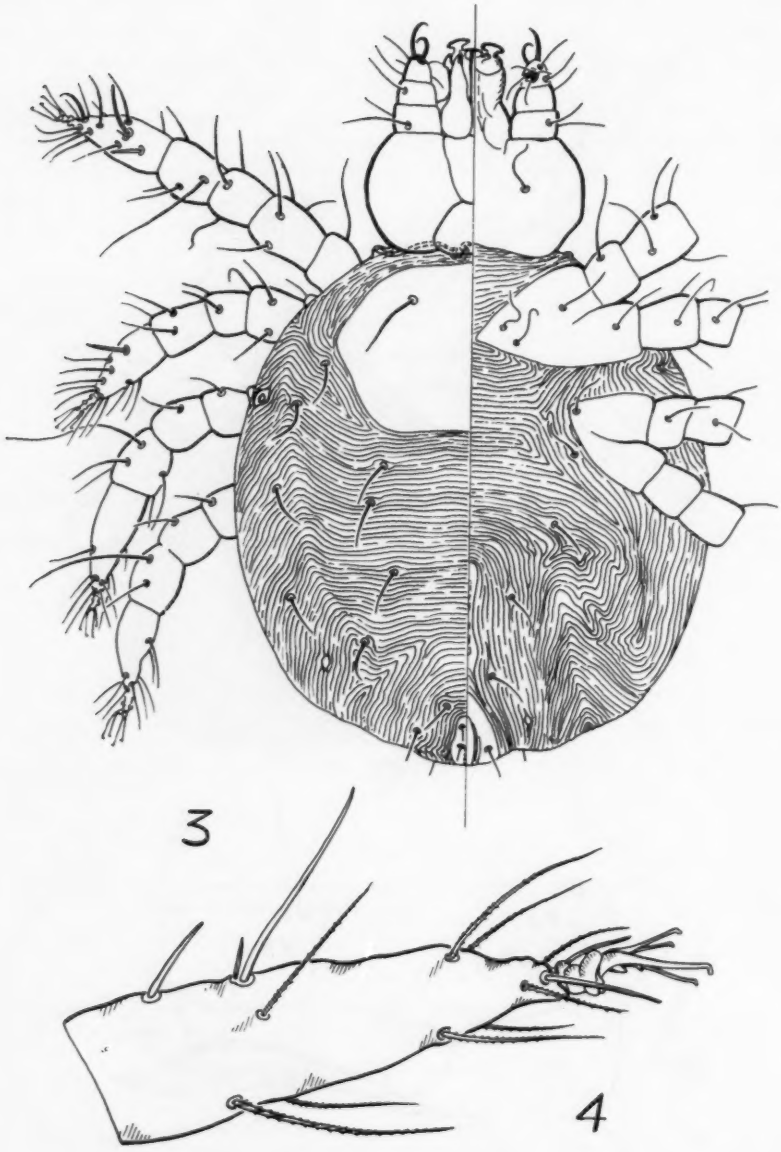


PLATE III

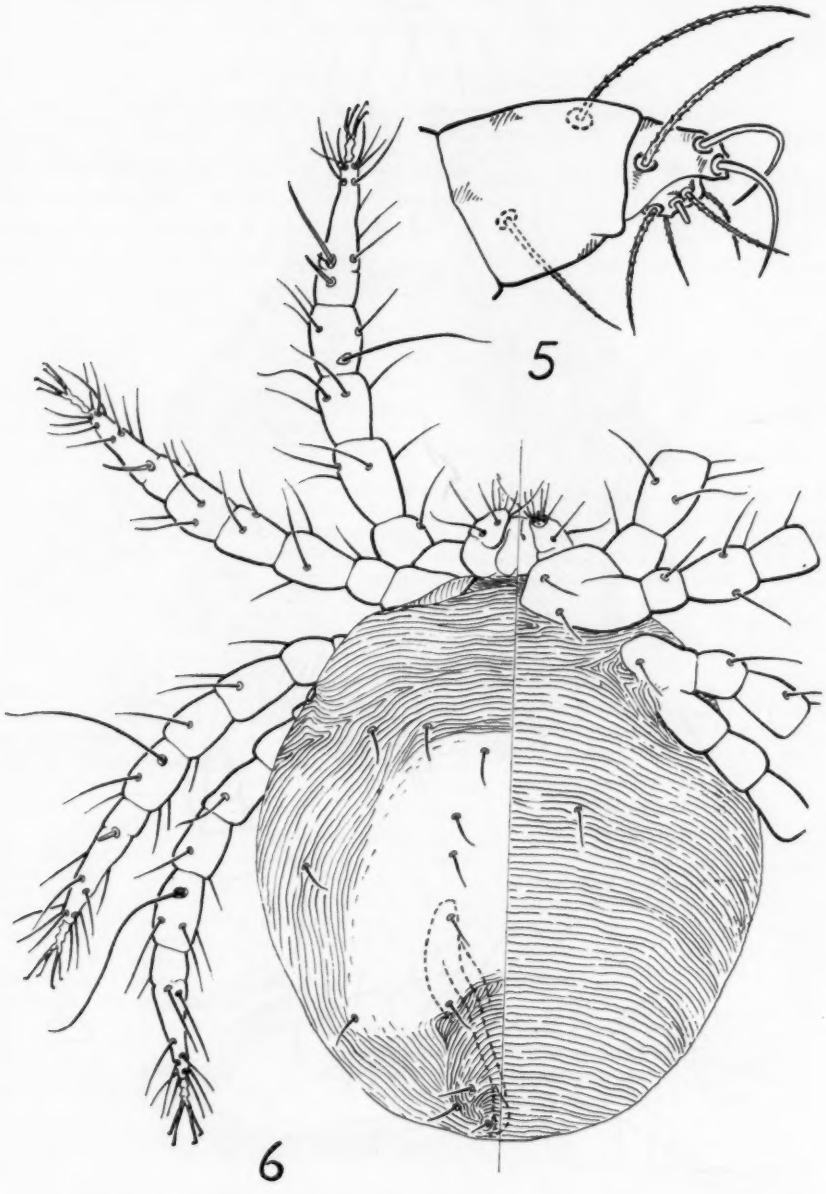
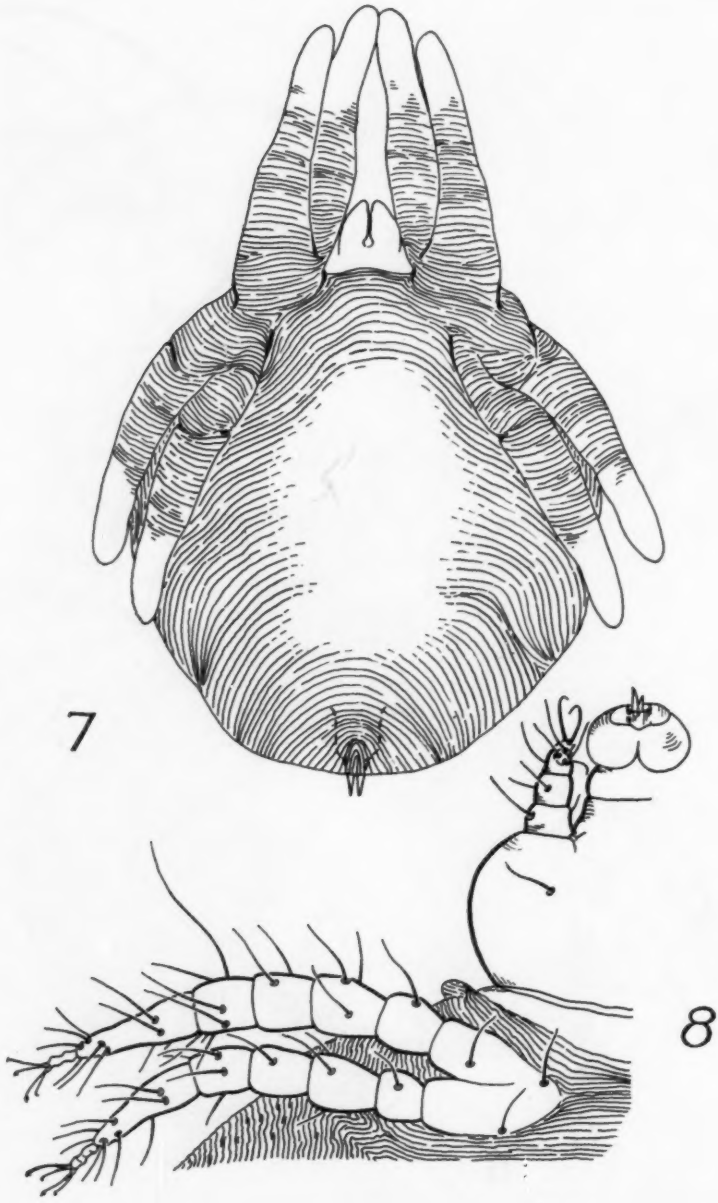


PLATE IV



BOOPHILUS KOHLSI N. SP. (ACARINA: IXODIDAE) FROM SHEEP AND GOATS IN JORDAN*

HARRY HOOGSTRAAL AND MAKRAM N. KAISER

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The three species recognized in the ixodid genus *Boophilus,* all renowned vectors of pathogens, infest chiefly cattle, horses, deer, and antelopes in rather humid tropical and subtropical areas throughout the world. The finding of a fourth species, described below, with an apparent predilection for sheep and goat hosts and in a comparatively arid habitat is, therefore, of special interest.

Boophilus kohlsi n. sp.
Kohls' Jordanian Sheep and Goat Boophilid
(Figs. 1 to 18)

Holotype. Male, from domestic goat, Irbid, Irbid, Jordan, 13 October 1958. Deposited in the United States National Museum.

Allotype. Female, same data as holotype. Deposited in the United States National Museum.

Paratypes. Total: 12 males, 83 females, 50 nymphs. *From domestic goats:* 1 female, 4NN, Tarkomia, Hebron, 6 September 1958. 6 females, Alsulaime, Salt, 15 September 1958. 1 male, 2 females, 18NN, Irbid, Irbid, 13 October 1958. 6 females, Akraba, Nablus, 11 October 1958. 7 females, Betta, Nablus, 12 October 1958. 3 males, 9 females, 24NN, Toupas, Nablus, 6 October 1958. *From domestic sheep:* 12 females, Alsulaime, Salt, 15 September 1958. 7 females, Betonia, Ramallah, 7 October 1958. 1 male, Alzahrieh, Hebron, 6 September 1958. 3 males, 6 females, Albadia-Shamalleh, Mafraq, 8 October 1958. 1 female, Alkarm, Jerash, 26 September 1958. 3 males, Alsawieh, Nablus, 2 October 1958. 2 males, 6 females, 2NN, Betta, Nablus, 12 October 1958. 3 males, 16 females, 2NN, Toupas, Nablus, 6 October 1958.

Paratypes are deposited in collections of the United States National Museum, British Museum (Natural History), Chicago Natural History Museum, Rocky Mountain Laboratory, Division of Veterinary Services (Onderstepoort), East African Veterinary Research Organization, Department of Zoology (University of Maryland), United States Department of Agriculture (Beltsville), and the writers.

Description

MALE (figs. 1 to 6). Robust, measuring from 2.016 mm to 2.400 mm in length (from apex of papli to apex of caudal appendage) and from 1.332 mm to 1.548 mm in width at widest point of body. Color reddish to yellowish brown in alcohol preserved specimens.

Capitulum (figs. 3, 4). *Basis capituli* slightly more than twice as wide as long; lateral margins either very slightly undulating but subparallel or mildly convex; posterior margin straight or slightly concave; cornua widely triangular, rounded posteriorly; surface posterior of hypostome devoid of bristles, bearing 18 or 19 bristles in each lateral field and an anterolateral tuft of approximately 8 bristles. Ventral margins slightly convex basally, diverging anteriorly, slightly recurved towards base of palpi; ventral surface with approximately the same number of bristles as dorsal surface but these distributed in 1 anterior group posterior of palpi and 1 group of shorter bristles in posterolateral corners; a pair of very short posthypostomal bristles arising level with base of palpal segment 1. *Hypostome* equalling palpi in length; apex blunt (rarely slightly indented) and bearing a corona of minute hooklets; lateral outline

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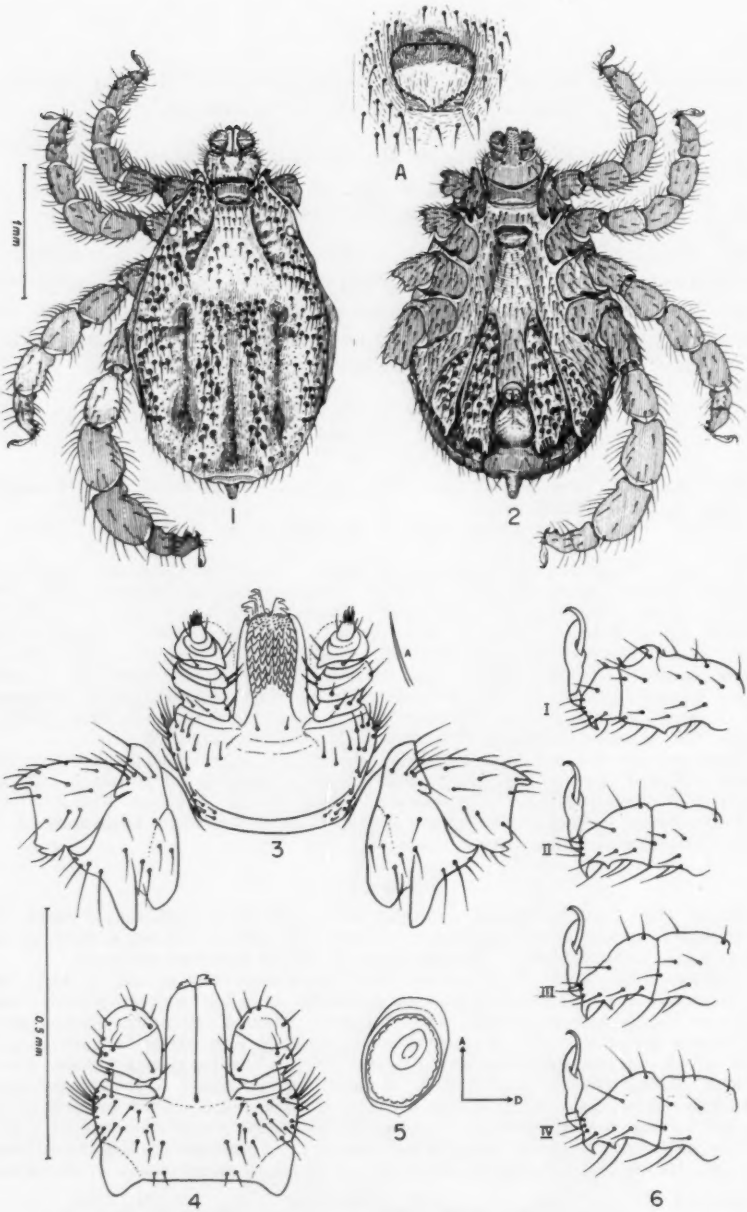


FIGURES 1 to 6. *Boophilus kohlsi* n. sp., male: 1 and 2, dorsal and ventral views; 2A, genital area (enlarged); 3, capitulum (ventral view) and coxae I; 3A, bristle of ventral lobe of palpal segment 1 (enlarged); 4, capitulum, dorsal view; 5, spiracular plate; 6, tarsi I to IV, lateral view.

narrow basally, slightly diverging anteriorly; dentition 4/4; outer file of approximately 9 denticles, inner files of 8, 7, and 6 denticles. *Palpi* with broadly rounded apex; lateral margins may vary slightly (compare right and left margins, fig. 4). Segment 1 visible ventrally, narrower than 2 or 3; a conspicuous lobe bearing a large, wide bristle (figs. 3 and 3A) arising from inner margin; 3 narrow bristles on ventral surface laterad of lobe. Segment 2 dorsally with outer margin narrow and inner margin wider; ventral margins more nearly equal; ventral surface bearing a mild spurlike lobe from the inner margin of which 2 wide bristles arise. Segment 3 ventrally bearing a more definite and pointed inner spur, bearing 2 narrow bristles on inner margin. Segment 4 arising ventrally from apex of segment 3.

Scutum measuring from 1.836 mm to 2.052 mm in length (from apex of scapulae to apex of caudal appendage) and from 1.116 mm to 1.188 mm in width. Posterior margin gradually to bluntly rounded. Lateral margins slightly diverging to level of coxae IV, thence gradually more acutely converging to scapulae; bordered by a narrow external margin of integument that expands somewhat during feeding and widens the general impression of body width in area of midlength of scutum. Scapulae narrowly triangular, anterior emargination deep. (Anterior spurs of coxa I visible dorsally). A short, wide, truncate caudal appendage present. *Eyes* small, subcircular in outline, flat or very slightly convex; often inconspicuous; situated at level of coxa II. *Cervical grooves* either shallow or deep, diverging, extending to level of coxa III. *Posteromedian groove* deep; *paramedian grooves* longer than posteromedian groove. *Punctations* large, deep, bristle-bearing depressions regularly distributed in areas between grooves and margins (fig. 1).

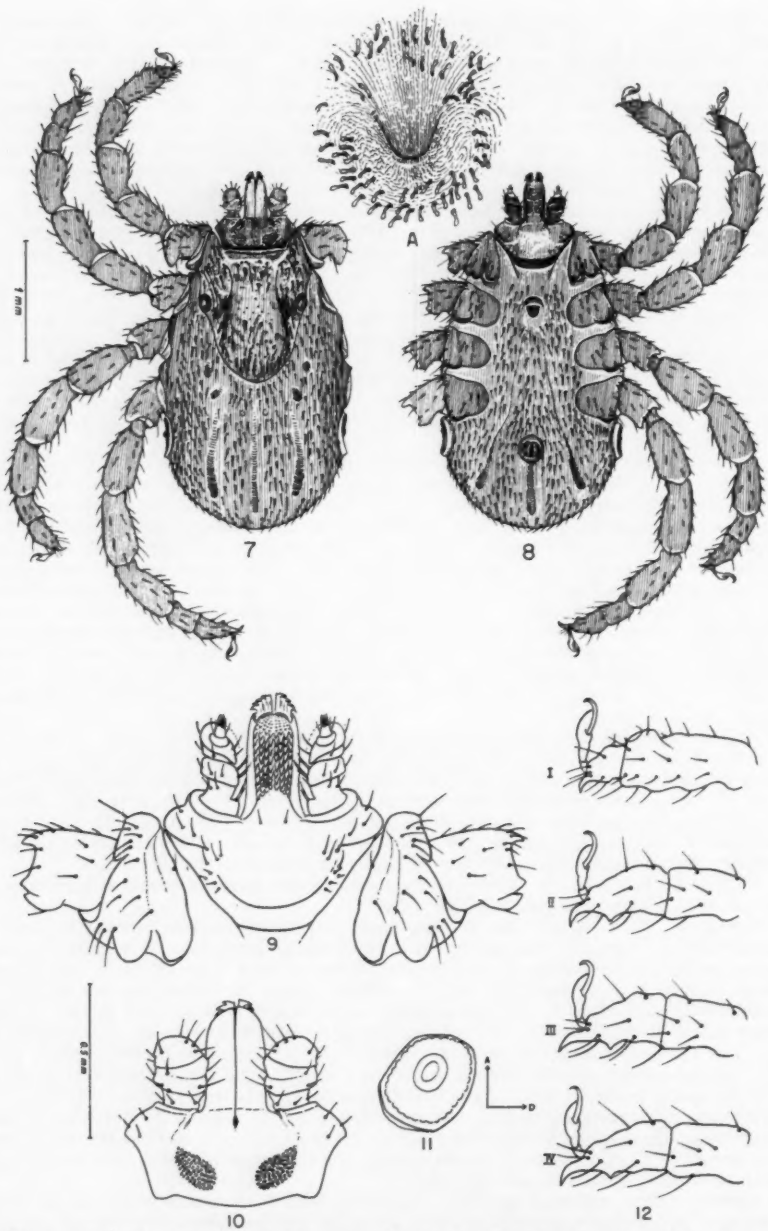
Spiracular plate subcircular (fig. 5). *Genital area* situated level with anterior half of coxa II (fig. 2). Apron short and wide; posterior margin with small, irregular serrations (fig. 2A). *Genital grooves* diverging from genital area to bound lateral margins of adanal shields. *Adanal shields* commencing at midlevel of coxae IV and extending almost to posterior body margin (not exceeding this margin); outline narrowly elongate, posterior margin deeply concave between a short outer spur and a longer inner spur; outer margin convex; inner margin practically straight to level of anus, thence slightly concave to apex; surface with numerous large, bristle-bearing punctations. *Accessory shields* approximately 0.75 as long as adanals, subtriangular; distally same distance from posterior margin of body as adanals; bearing a terminal spurlike projection intermediate between those of the adanals in size; surface bearing punctations and bristles similar to those of adanals. *Anus* subcircular; at least 2 bristles arising from each valve. *Integumental setae* moderately numerous, distributed as illustrated (fig. 2).

Legs comparatively long and robust, shape normal for species in this genus. *Coxa* I (fig. 3) with a large anterior spur visible from dorsal view; posteriorly deeply cleft to form a bluntly rounded inner spur and a narrower, slightly longer, more pointed outer spur. Coxae II and III with a small, ridgelike outer spur and a suggestion of a spur at juncture of inner and posterior margins; these even less developed on III than on II. Coxae IV with a very small, widely subtriangular, median spur. *Trochanter* I dorsally with a comparatively large, triangular shield. *Tarsi*, *claws*, and *pads* (pulvilli) as illustrated (fig. 6).

FEMALE (figs. 7 to 12) The least engorged available specimen measures approximately 2.90 mm in length and 1.68 mm in width.

Capitulum. *Basis capituli* (fig. 10) hexagonal with lateral margins forming an angle at anterior third of length; cornua merely slight subtriangular projections of margins; posterior margin convex; surface bearing 3 short bristles confined to area of lateral angle; porose areas shallow, moderately small, apparently usually pyriform (appear to be oval in a few specimens), separated from each other by an area equalling approximately 0.75 of their length. Ventral surface ridged as illustrated (fig. 9) and bearing approximately 10 pairs of sublateral bristles and 1 pair of posthypostomal bristles. *Hypostome* (fig. 9) somewhat narrower than that of male, slightly notched apically, with a larger corona, and with slightly narrower denticles; otherwise similar to that of male. *Palpi* dorsally essentially like those of male. Ventrally with lobe of segment 1 forming a projecting base and lacking a lobe or ridge on 2; 3 like that of male; a single wide, frayed bristle arising from inner basal lobe of 1; 2 or 3 such bristles arising from inner margin of 2; two narrower bristles arising from inner margin of 3; one or 2 narrow bristles on ventral surface of each segment.

Scutum of least engorged specimens measuring from 0.900 mm to 0.972 mm in length (from apex of scapulae to posterior margin) and 0.756 mm (exceptionally 0.936 mm) at point of greatest width. Lateral margins slightly diverging and thence converging to form a blunt angle at mid-distance between apex and eyes; margins posterior of eyes gradually converging. Cervical grooves concave, reaching posterolateral margin. Surface usually rugose except in central area, with few punctations of mixed sizes and very few bristles; approximately 12 large bristle-bearing punctations in each lateral field anterior of eyes and 10 or 12 similar ones



FIGURES 7 to 12. *Boophilus kohlsi* n. sp., female: 7 and 8, dorsal and ventral views; 8A, genital area (enlarged); 9, capitulum (ventral view) and coxae I; 10, capitulum, dorsal view; 11, spiracular plate; 12, tarsi I to IV, lateral view.

between cervical grooves near anterior margin. *Eyes* bulging, elongately oval to more nearly subcircular in outline.

Dorsal integument with bristles and groovelike depressions as illustrated (fig. 7).

Spiracular plate subcircular (fig. 11). *Genital area* situated level with anterior half of coxae II (fig. 8). Genital apron (fig. 8A) with converging lateral margins and gradually rounded posterior margin in very slightly engorged specimens. *Anus,* and ventral bristles and furrows as illustrated (fig. 8).

Legs comparatively long and robust, shape normal for species in this genus. *Coxa* I (fig. 9) deeply cleft to form 2 subequal spurs the outer of which is slightly wider than the inner and slightly overlaps the posterior margin of the coxa. Coxae II to IV with progressively smaller posterior ridges on outer half of margin. *Trochanter* I dorsally with a comparatively large, triangular shield. *Tarsi, claws,* and *pads* (pulvilli) as illustrated (fig. 12).

NYMPH (figs. 13 to 18). Least engorged available specimens measure approximately 1.15 mm in length and 0.62 mm in width.

Capitulum. Basis capituli (figs. 15 and 16) with outlines as illustrated; ventrally size of anterior projection of lateral margins is variable; bristles confined to a pair of short posthypostomals and 2 other pairs on ventral surface. *Hypostome* wide, with diverging lateral margins, blunt and slightly indented apical margin, a large corona of hooklets, and 3/3 dentition of 7, 6 and 5 denticles from outer to inner row. *Palpi* ventrally with juncture between segments 1 and 2 somewhat obscure, a single wide, frayed bristle arising from inner margin of 2 and 3 (not from 1 and 2 as in adults).

Scutum measuring approximately 0.5 mm in length and 0.5 mm in width. Outline convex from apex to eyes, thence gradually converging to narrow posterior margin. *Cervical grooves* shallow, straight, parallel, confined to anterior half of scutal length. Surface slightly rugose laterally; with a pair of submedian bristle-bearing punctations and 4 or 5 sublateral bristle-bearing punctations anterior of eyes. *Eyes* small, flat, inconspicuous.

Dorsal integument (fig. 13) with approximately 21 lateral and sublateral bristles on each side posterior of eyes, and with 2 pairs of submedian bristles; also with a posteromedian and a pair of paramedian furrows.

Spiracular plate subcircular (fig. 17). *Genital grooves* extending from level of coxae II to anus. *Anus* subcircular, bearing a single bristle on each valve. *Ventral integument* with few bristles and with a postanal and pair of paramedian furrows (fig. 14).

Legs of size and shape normal for species in this genus. *Coxae* with posterior spurs gradually decreasing in size from I to IV, that of IV much reduced; those of II and III wider than that of I. *Tarsi, claws,* and *pads* (pulvilli) as illustrated (fig. 18).

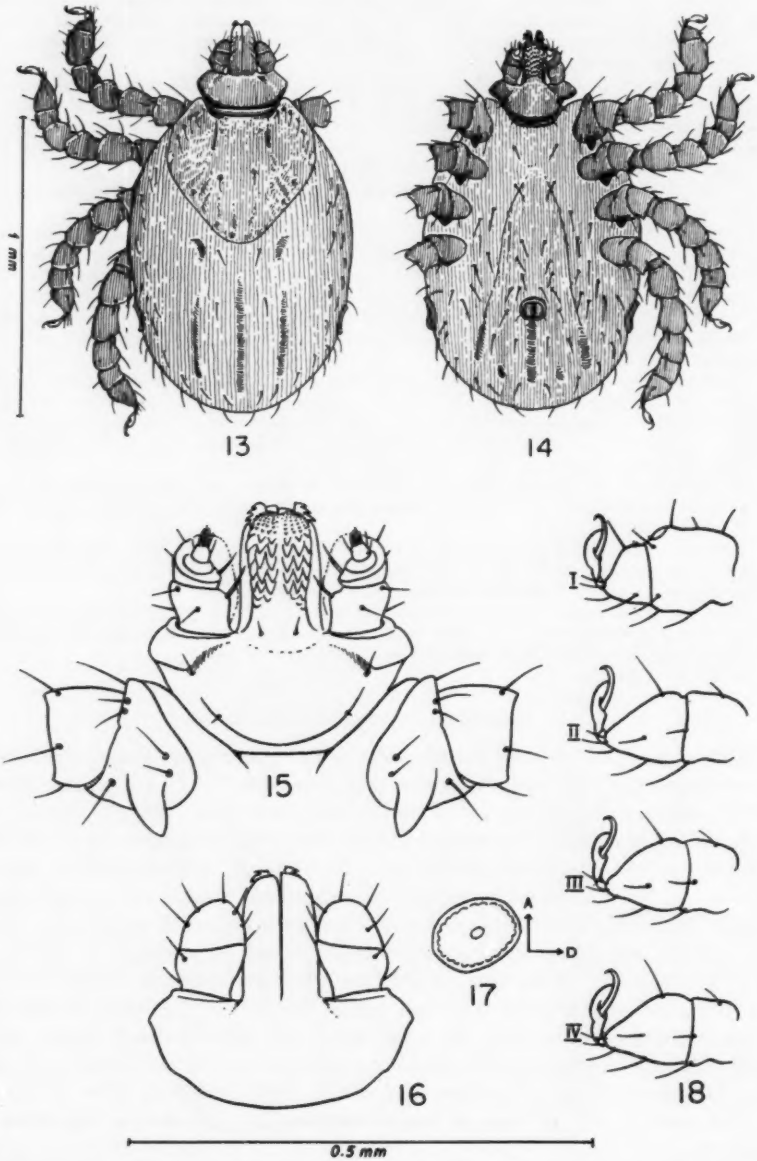
LARVA. Unknown.

DISCUSSION AND CONCLUSIONS

Related species. The only other species in this genus possessing a bristle-bearing protuberance on the inner margin of palpal segment 1 is *B. decoloratus* (Koch, 1844), a parasite of antelope, cattle, and equines in tropical Africa, which uncommonly infests sheep and goats and which requires a much higher relative humidity than that occurring anywhere in Jordan. Also like *B. decoloratus,* the male of *kohlsi* n. sp. bears a caudal appendage and its adanal shields are spurred posteriorly. The adanal shields of *kohlsi* n. sp., however, are much smaller than those of *decoloratus* and do not reach or overlap the ventral body margin as they do in the African species. In the new species these spurs are subequal, while in *decoloratus* the inner spur is many times the size of the outer. The shape of the male genital area and of the spurs of the male coxa I also differ in the 2 species. Most significantly, the dentition of both sexes of *decoloratus* is 3/3; in *kohlsi* n. sp., it is 4/4. The shape of the porose areas and of the genital area also differ in females of these species, and the eyes of female *kohlsi* n. sp., are larger than those of *decoloratus.*

Diagnosis. Both sexes with a bristle-bearing protuberance ventrally on the inner margin of palpal segment 1, and with 4/4 dentition. Male with caudal appendage. Adanal shields not overlapping posterior body margin; with a concave posterior margin between a larger inner and a smaller outer spur; accessory shields with an inner spur intermediate in size between

those of adanals. Genital apron of male short and wide; of female shield-shaped. Coxae I of both sexes deeply cleft. Porose areas pyriform, fairly small. Parasitizes chiefly sheep and goats in moderately arid area of Near East.



FIGURES 13 to 18. *Boophilus kohlsi* n. sp., nymph: 13 and 14, dorsal and ventral views; 15, capitulum (ventral view) and coxae I; 16, capitulum, dorsal view; 17, spiracular plate; 18, tarsi I to IV, lateral view.

Other species. The 2 other species presently recognized in the genus *Boophilus* (Hoogstraal, 1956, pp. 292 to 324) also have 4/4 dentition but lack a bristle-bearing protuberance on palpal segment 1. The male of *B. annulatus* lacks a caudal appendage and its female's coxae I are not cleft. Numerous other characters, such as genital aprons, porose areas, and form of ventral shields also differ. In *B. microplus* a caudal appendage is present but the genital and porose areas differ as do the ventral shields, etc. Both these species parasitize chiefly deer, cattle, and equines in more humid areas than *B. kohlsi* n. sp.

Hosts. *B. kohlsi* n. sp. is the only species in the genus for which sheep and goats, rather than cattle, horses, deer and antelopes, appear to be hosts of predilection. Our collections contain numerous specimens of *B. annulatus* from cattle and horses in Jordan but none of this species from sheep or goats.

Distribution. Closely related to *B. decoloratus* of the Ethiopian Faunal Region, *B. kohlsi* n. sp. is thus far known only from a few areas between the Sea of Galilee in the north and the Dead Sea in the south of Jordan. We suspect that it might range into northern Syria, from where a specimen of *"B. decoloratus"* was reported by Minning (1934). *Boophilus* ticks are often widely distributed as a result of movements of domestic animals. Once criteria for this species are recognized, it is expected that populations might be found in different areas.

Economic importance. The other 3 species recognized in the genus *Boophilus* are notorious vectors of animal diseases in all continents of the world. The epidemiological role of *B. kohlsi* n. sp. should, therefore, be well worth investigation. This new species was discovered as a result of a collaborative effort between medical and veterinary organizations in northern Africa and the Near East to widen the scope of knowledge concerning ticks of domestic animals in this area. A basic factor in ameliorating human health problems in developing countries is improvement of the health and vitality of domestic animals and increasing meat supplies available to all the population.

Participants in this program are veterinarians of various governments and specialists of the Animal Production Branch of the Food and Agriculture Organization of the United Nations (FAO), and the Medical Zoology Department of the U. S. Naval Medical Research Unit Number 3. The specimens described below were collected by members of the Veterinary Department of the Ministry of Agriculture, Jordan, and forwarded to the writers by Dr. Kamel El Taher, Director General.

Dedication. *B. kohlsi* n. sp. is named for Mr. Glen M. Kohls, Sanitarian Director, Rocky Mountain Laboratory, Hamilton, Montana, not only because of his many kindnesses and considerable assistance to the writers over a period of years, but chiefly in recognition of his enormous contributions to knowledge of ticks of the world during the past quarter of a century.

SUMMARY

Boophilus kohlsi n. sp., described from males, females, and nymphs from Jordan, is morphologically related to *B. decoloratus* of tropical Africa. Unlike other boophilids, this species appears to prefer sheep and goats as hosts and inhabits a comparatively arid area of the world.

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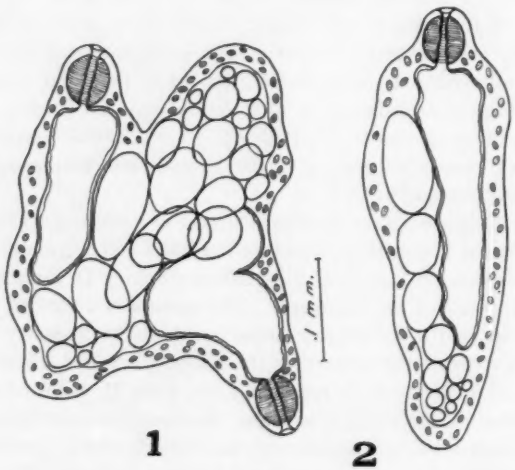
RESEARCH NOTE

A CASE OF CONJOINED TWIN REDIAE.

During the summer of 1959 the author examined a considerable number of pond snails in search of natural infections of a certain plagiorchioid trematode. One specimen of *Physa gyrina* Say harbored a monostome infection in which approximately 40 well-developed rediae and a small number of cercariae had been produced. Among the rediae was 1 pair of conjoined twins. The oral openings of the twins (fig. 1) were diametrically opposed. Each twin contained a fully developed muscular pharynx and an intestine. The body cavity, containing numerous cercarial embryos in different stages of development, was shared by the conjoined pair. The dimensions of the pair were 0.343 mm long by 0.280 mm wide. A normal monostome redia is illustrated in Figure 2 for comparison. The figures are from living specimens drawn to the same scale with the aid of a camera lucida.



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The cercariae from the snail were placed on lettuce leaves for encystment. After approximately 24 hours the lettuce was fed to an albino laboratory rat for production of the adult trematode. The rat was autopsied 31 days later at which time 17 sexually mature specimens of *Notocotylus quinqueserialis* Barker and Laughlin were found. The snails had been collected originally from Robinson's Lake, Latah County, Idaho. The above species of trematode is a common parasite of muskrats in the vicinity of this lake.

To date, only a few papers have been published which deal with abnormalities among the larval stages of trematodes. Kuntz (1948, Proc. Helm. Soc. Wash. **15:** 73–77) has ably reviewed all previously published literature on this subject. It seems that abnormalities, in the form of partial twinning, have been restricted entirely to the miracidial and cercarial stages. The author believes that this is the first report of an abnormality of this type in a redia.—STEWART C. SCHELL, *Department of Biological Sciences, University of Idaho, Moscow, Idaho.*

OBSERVATIONS ON THE LIFE HISTORY OF THE BAT TICK *ORNITHODOROS KELLEYI* (ACARINA: ARGASIDAE)

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Several species of soft ticks are known to occur only on bats or in bat retreats, but our knowledge concerning the biology of most of these is incomplete and for some no information is available. One about which relatively little is known is *Ornithodoros kelleyi* Cooley and Kohls, 1941. The published information about this species consists of the descriptions of the larva, the second stage nymph, and the female (Cooley and Kohls, 1941, 1944) and brief notes on its feeding and molting habits (Davis, 1942; Anastos and Clifford, 1956). This tick has a very wide geographical distribution extending from New York to Utah; yet, relatively few specimens have been collected and all of these have been taken from bats, in caves, and in human habitations frequented by bats. It has been recorded in the United States from Colorado, Illinois, Iowa, Minnesota, New York, Pennsylvania, Wisconsin, Utah (Cooley and Kohls, 1944), Ohio, West Virginia (Bequaert, 1946), Maryland (Anastos and Clifford, 1956), and Indiana (Wilson, 1958) and in Canada from Saskatchewan (Gregson, 1956).

A large breeding colony of *O. kelleyi* established in the laboratory from 154 specimens collected in the cracks and crevices of the walls of a church attic in Chaptico, St. Mary's County, Maryland, in 1954 and 1955 has made possible certain studies which have increased our knowledge of the biology of all stages in the life cycle.

MATERIALS AND METHODS

Early in the course of this study, the inability to keep bats alive for several days until the larvae had completed their feeding necessitated trying white rats and guinea pigs as possible hosts for this stage. The larvae fed readily on these rodents, especially on rats, but the nymphs and adults would not feed on any hosts other than the bats *Eptesicus fuscus* Beauvois, *Pipistrellus subflavus* (Cuvier), *Corynorhinus rafinesquii* (Lesson), and *Myotis lucifugus* (Le Conte). Later, methods were developed which lengthened the survival time of the bats so that the larvae, as well as the subsequent developmental stages, could be fed exclusively on them.

All stages of *O. kelleyi,* including the egg, were stored in a constant temperature cabinet maintained at 30 C and 77 percent relative humidity. The feeding of the ticks was done at room temperature and in darkened chambers. Larvae which were fed on rodents were placed into chambers made from the screw tops and covers of 1.25-inch mailing tubes taped onto the shaven abdomen of the host. Larvae and all of the other stages which fed on bats were placed into 0.5-pint cylindrical containers containing the host. Each tick when it had detached from the host, following the completion of feeding, was placed in a serially numbered cotton-stoppered vial and returned to the storage cabinet.

RESULTS AND DISCUSSION

No differences were found in the general aspects of the biology between the ticks reared on a combination of experimental and normal hosts and those reared solely on their normal host, but some minor differences were observed in the rate of feed-

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ing and in total developmental time. Whether these differences can be attributed to the use of unnatural hosts cannot be settled at this time since additional data are required. For this reason the specific information acquired for these 2 groups of ticks concerning feeding time, molting time, mating, oviposition, and hatching are treated separately.

The life cycle of *O. kelleyi* was found to consist of egg, larva, 2 to 4 nymphs, and adult male or female. Under laboratory conditions the minimum time for the completion of the cycle was 54 days; maximum, 258 days. The hexapod larva after a long feeding period, followed by a quiescent period, molted into the 1st nymphal stage with the characteristic 8 legs. The 1st nymph did not feed and molted into the 2d nymphal stage following a short resting period. The occurrence of a non-feeding 1st nymph in the life cycle has been reported also for *O. talaje* (Guérin-Méneville), *O. concanensis* Cooley and Kohls, *O. coriaceus* Koch (Davis, 1942), *O. puertoricensis* Fox, *O. dugesi* Mazzotti (Davis, 1955), and *O. coniceps* (Canestrini) (Davis and Mavros, 1956). The 2d nymphal stage resumed the feeding activity and, following engorgement and a resting period, it molted either into the 3d nymphal stage or into the male stage. No females were observed to issue from the 2d nymphal stage and it appeared that a definite tendency exists for the males to mature earlier than the females. The 1st appearance of the female was from the 3d nymph; but this nymph, following engorgement and a quiescent period, also gave rise to the 4th nymphal stage as well as to the male stage. The 4th-stage nymph, however, after feeding and resting gave rise to the female stage only.

Feeding habits (tables I and II). The recorded hosts for *O. kelleyi* are the

TABLE I. *Feeding times of* Ornithodoros kelleyi *larvae.*

Days required for engorgement	Number of larvae fed on bats	Number of larvae fed on white rats
8	0	7
9	1	17
10	1	26
11	2	33
12	8	24
13	2	23
14	4	20
15	5	15
16	4	6
17	3	8
18	2	2
19	5	0
20	7	0
	44	181

bats *Myotis californicus pallidus* Stephens, *Myotis lucifugus* (Le Conte), *Pipistrellus subflavus* (Cuvier), *P. h. hesperus* (Allen) (Cooley and Kohls, 1944) and *Eptesicus f. fuscus* (Beauvois) (Bequaert, 1946). Davis (1942) reported that *O. kelleyi* as well as 3 other bat ticks, viz, *O. dunni* Matheson, *O. concanensis* Cooley and Kohls, and *O. stageri* Cooley and Kohls, would feed readily on small laboratory animals, especially in the nymphal and adult stages. In the present study it was found that the larvae of *O. kelleyi* would feed successfully on bats and white rats but less satisfactorily on guinea pigs. However, the nymphal stages and the adults would feed only on bats. All stages appeared to prefer subdued light or darkness to begin the feeding activity.

The larvae in contrast to the nymphs and the adults were relatively slow feeders and required several days to feed to repletion. The 1st nymphal stage was a non-

TABLE II. *Feeding times of the adults and nymphal stages of* Ornithodoros kelleyi.

Feeding time in minutes	Number of ticks fed on bats in all stages, including the larval stage				Number of ticks fed on white rats in the larval stage and on bats in all other stages				
	2d N	3d N	M	F	2d N	3d N	4th N	M	F
20– 24					3				
25– 29					2			3	1
30– 34	1				12	1	1	5	0
35– 39	0	1	1		14	1	0	5	1
40– 44	5	0	0		11	0	1	1	1
45– 49	8	1	2		9	2	0	1	1
50– 54	7	2	2	2	6	2	2	2	3
55– 59	3	1	0	0	3	3	0	1	1
60– 64	5	1	1	1	7	10	0	2	4
65– 69	1	3	1	0	1	9	0	1	4
70– 74	4	2	1	2	5	3	0	1	4
75– 79	2	1	1	0	2	5	2	0	2
80– 84	1	3	1	0		1	0	2	5
85– 89	1	1	0	0		5	0	3	2
90– 94		2	0	0		6	0	0	1
95– 99		1	0	0		1	1	3	4
100–104		2	0	0		3	0	1	4
105–109		3	0	0		5	1	0	3
110–114		0	0	1		5	0	0	2
115–119		0	1	0		6	0	2	3
120–124		2		0		2	2	1	1
125–129		0		0		3	1	0	2
130–134		0		1		2	1	0	0
135–139		2				1	0	1	1
140–144						1	1	1	0
145–149							1		1
150–154									1
	38	28	11	7	75	77	14	36	52

feeding form and did not feed even though given the opportunity to do so. The other nymphal stages and the adults completed their feeding in 20 to 150 minutes. The males of *O. kelleyi* became as fully distended as the females or any of the other developmental stages. Coxal fluid was not given off by the larval stage during or after feeding, but it was observed during the feeding of the nymphs and the adults.

The feeding time for 44 larvae which fed to repletion on 1 large brown bat, *E. fuscus,* was 9 to 20 days (mean, 15.4); for 181 larvae which completed engorgement on white rats it was 8 to 18 days (mean, 12.2). A total of 104 attached to 12 guinea pigs, but the mortality among these larvae was extremely high, since only 3 of them survived. A rash developed at the sites of attachment of all these larvae feeding on guinea pigs, but this reaction was not observed in any of the other hosts used; possibly it represents a case of host immunity to ticks.

Feeding times for nymphs and adults reared from larvae fed on bats were: 38 second-stage nymphs, 30 to 85 minutes (mean, 54.7); 28 third-stage nymphs, 37 to 135 minutes (mean, 84.2); information on the feeding time of 1 fourth-stage nymph was not obtained; 10 males, including 1 which fed twice, 35 to 115 minutes (mean, 63.3); and 7 females, 50 to 131 minutes (mean, 78.3).

Feeding times for nymphs and adults reared from larvae fed on rats were: 75 second-stage nymphs, 20 to 75 minutes (mean, 44.1); 77 third-stage nymphs, including 10 which fed twice, 32 to 144 minutes (mean, 86.4); 14 fourth-stage nymphs, including 2 which fed twice, 33 to 148 minutes (mean, 94.6); 36 males, including 5 which fed twice, 27 to 140 minutes (mean, 66.1); and 36 females, including 15 which fed twice and 1 which fed 3 times, 25 to 150 minutes (mean, 86.2).

Molting habits (table III). A single molt occurred for each stage until the adult stage was reached, and the adults were never observed to molt after reaching this stage even though subsequent feedings occurred. In most cases a single feeding was sufficient for the various nymphal stages to proceed to the next stage, but 10

third-stage nymphs reared from larvae fed on rats required a 2d feeding before molting. It is well known that the interval of time between the completion of feeding and the occurrence of molting in argasid ticks may be shortened by an elevation of temperature or be delayed by a lowering of the temperature. Probably a similar situation exists in *O. kelleyi,* but this was not determined in the present study since all ticks were kept at a uniform temperature, 30 C. It was observed, however, that the time interval required for molting between successive developmental stages became progressively longer, *i.e.,* the larval molt required less time than the final molt to reach the adult stage.

TABLE III. *Elapsed time in days between feeding and molting for the larval and nymphal stages of* Ornithodoros kelleyi.

Days	Number of ticks fed on bats in all stages, including the larval stage				Number of ticks fed on white rats in the larval stage and on bats in all other stages				
	L	1st N	2d N	3d N	L	1st N	2d N	3d N	4th N
2	1				9				
3	14				47				
4	19				67				
5	5				17				
6	1				5				
7									
8		2				4			
9		2				6			
10		6				14			
11		10				31	1		
12		7	7			24	3		
13		4	4			24	13		
14		6	9			19	18	3	
15		1	6	1		6	16	1	
16		3	3	0		6	19	1	1
17			4	2		3	16	8	0
18			2	2		2	2	10	0
19			0	2			4	11	2
20			1	3			1	8	1
21			0	9			5	9	1
22			1	2			1	2	0
23			0	3			0	3	0
24			1	0			1	0	0
25			0	0			0	0	1
26			1	1			1	1	1
27				0			0	0	0
28				0			1	3	1
29				0				0	1
30				0				2	0
31				1				1	1
32				0				0	
33				1				0	
34				0				0	
35				0				1	
36				0				0	
37				0				1	
38				0					
39				0					
52				1					
78									1
	40	41	39	28	145	139	102	65	11

The interval between the completion of feeding and the molt into the next stage for the specimens reared from larvae fed on bats was: 40 larvae to 1st-stage nymph, 2 to 6 days (mean, 3.8); 41 first-stage nymphs to 2d-stage nymphs, 8 to 16 days (mean, 12.1); 39 second-stage nymphs to 10 males and 29 third-stage nymphs, 12 to 26 days (mean, 15.3); 28 third-stage nymphs to 8 males, 19 females and 1 fourth-stage nymph, 15 to 52 days (mean, 22.4); and 1 fourth-stage nymph to female, 29 days. Similarly, the interval for those reared from larvae fed on rats was: 145 larvae to 1st-stage nymphs, 2 to 6 days (mean, 3.8); 139 first-stage nymphs to 2d-stage nymphs, 8 to 18 days (mean, 10.9); 102 second-stage nymphs to 14 males and 88 third-stage nymphs, 11 to 28 days (mean, 15.9); 65 third-stage nymphs, including 10 which had a 2d feeding, to 30 males, 30 females and 15 fourth-stage nymphs, 14

to 37 days (mean, 20.1); and 11 fourth-stage nymphs to females, 16 to 78 days (mean, 28.1).

Copulation, oviposition and hatching (table IV). The observed mating time for 5 pairs of *O. kelleyi* was 20 to 55 minutes from the immobilization of the attached male to the time of separation. Copulation occurred prior to feeding for 3 of these pairs, and after feeding in the other 2 pairs. Only 1 of these females, one which had copulated prior to feeding, eventually oviposited.

Other females were provided with a male upon the completion of feeding, but since they were returned immediately to the storage cabinet, copulation was not observed. Oviposition for this group of females occurred 10 to 210 days after the completion of feeding. It is not known if the long delay in oviposition exhibited by some of the females represents another instance of a winter resting period from oviposition as reported for *O. parkeri* by Davis (1941), since it is not known if copulation occurred immediately after feeding or whether it was delayed for a protracted period of time.

Table IV shows the date of engorgement, the beginning of oviposition, and the

TABLE IV. *Oviposition and hatching data for* Ornithodoros kelleyi.

Date of Engorgement	Ticks fed on bats in all stages, including the larval stage				Date of Engorgement	Ticks fed on rats in the larval stage and on bats in all other stages			
	First eggs	Total eggs	First larvae	Total larvae		First eggs	Total eggs	First larvae	Total larvae
11/16/58	4/10/59	30	4/21/59	21	11/ 2/57	3/30/58	25	4/13/58	25
11/20/58	4/ 8/59	37	4/19/59	30	12/ 2/57	3/22/58	33	4/ 3/58	33
12/ 6/58	4/ 8/59	28	4/21/59	28	12/26/57	7/11/58	52	7/22/58	48
12/ 7/58	3/28/59	75	4/18/59	75	6/ 5/58	6/22/58	28	7/ 4/58	28
2/23/59	4/ 5/59	55	4/18/59	55	6/ 5/58	6/23/58	61	7/ 2/58	61
2/23/59	4/ 8/59	63	4/18/59	60	6/15/58	7/ 4/58	26	7/16/58	26
2/23/59	4/ 7/59	45	4/21/59	40	6/16/58*	11/10/58	24		0
2/23/59	5/12/59	20	5/24/59	5	10/27/58	11/17/58	15		0
2/23/59	5/12/59	25	5/24/59	25	10/27/58	11/10/58	35		0
2/23/59	4/ 8/59	60	4/19/59	60	10/27/58	11/12/58	14		0
3/29/59	4/10/59	70	4/21/59	60					
3/29/59	4/ 8/59	65	4/19/59	65					
3/29/59	4/ 8/59	47	4/19/59	42					
3/29/59	4/ 8/59	38	4/23/59	38					
3/29/59	4/ 8/59	92	4/19/59	92					

* Not mated until 133 days after feeding.

appearance of the 1st larvae, with the total number of eggs and larvae for 15 females reared through all stages on bats and for 10 females reared from larvae on rats.

SUMMARY

Data are given on the distribution and natural and experimental hosts for the argasid tick *Ornithodoros kelleyi* found in bat retreats in Maryland. Observations are presented on feeding habits, molting habits, copulation, oviposition and hatching.

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Sincere appreciation is expressed to Dr. David H. Johnson of the U. S. National Museum for the identification of some of the bats used in this study and to Dr. Carleton M. Clifford and Dr. Conrad E. Yunker of the University of Maryland for some of the collections of *O. kelleyi*.

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RESEARCH NOTE

INDUCED HATCHING OF OPERCULATE EGGS.

During studies on *Fascioloides magna*, the hatching of large numbers of eggs at will was desired to provide miracidia. A method producing this result and fulfilling experimental needs has now become a successful routine procedure and may be of general use.

Eggs obtained from both bovine and cervine livers in Minnesota were washed by sedimentation through columns of distilled water (chlorinated tap water appeared to be toxic) until freed of debris. When stored under a few centimeters of distilled water at 5 C with air available, about half remained viable for over 1 year, and some even after 2.

Eggs in a single layer were allowed to embryonate in a depression slide (Maximov) containing 2 ml of distilled water. Such slides, kept in petri dishes containing water to retard evaporation, were incubated in the dark at room temperature. The time necessary varied, but at 25 C, miracidia appeared in many eggs within the 4th week. Although spontaneous hatching would occur slowly, they could usually be stored embryonated for weeks under these conditions.

Up to 80 percent of the embryonated eggs would hatch when placed in a bell jar under reduced pressure of 5 to 15 centimeters of mercury in the light at room temperature for over 60 minutes. Similar results were obtained by exposure to cylinder nitrogen (Ohio Chemical and Surgical Co. Water-Pumped Nitrogen, specified to be 99.5 percent pure and to contain traces of oxygen) at atmospheric pressure in the light for over 60 minutes.

Light alone did not induce simultaneous hatching as reliably as did reduced pressure or nitrogen. A pressure differential between the inside and the outside of the shell may possibly aid eclosion. *Diphyllobothrium latum* eggs, embryonated under 15 cm of water, were reported by Vogel (1929, Z. Parasitenk. **2:** 213–222) to hatch in large numbers when transferred to shallower water. This was attributed to a decrease in external pressure with a corresponding relative internal increase aiding displacement of the operculum. Dr. F. G. Wallace (University of Minnesota, personal communication) has found that exposure of *D. latum* eggs to a partial vacuum also induced their hatching. That reduced pressure in itself may not be the actual stimulus for *F. magna* eggs is suggested by the effect of cylinder nitrogen. Use of this gas as well as a vacuum would be expected to alter concentrations of atmospheric gases usually available. Partial anaerobiasis, therefore, may possibly play a role in the activation of the miracidium and be of general significance as a hatching stimulus for operculate eggs.—FRANK E. FRIEDL, *Department of Zoology, University of Minnesota.* (Present address: *The Rockefeller Institute, New York 21, New York.*)

FURTHER STUDIES ON HELMINTHS OF THE OPOSSUM, *DIDELPHIS VIRGINIANA,* WITH A DESCRIPTION OF A NEW SPECIES FROM THIS HOST.

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Marsupial parasites have been subjected to extensive investigation throughout the Americas. In North America, however, helminthological surveys of the opossum, *D. virginiana,* have been comparatively limited. Such studies include those of Dikmans (1931, Louisiana), Chandler (1932, Texas), Reiber and Byrd (1942, Tennessee), Byrd, Reiber, and Parker (1942, Tennessee, Georgia, and Illinois), and Babero (1957, Illinois). As a result of these investigations, considerable information relative to the bionomics of certain parasites and their occurrence and prevalence in marsupial hosts has been accumulated. For the most part, helminths of the opossum (*Didelphis* spp.) do not seem to be limited to a given geographical region since many of them frequently have been reported from animals that were inhabitants of widely separated areas. However, because of the endemicity of certain helminths of the opossum, additional studies in this regard seem warranted in order to provide further information concerning their geographical distribution, ecology, and host-parasite relationships.

During routine faunal investigations, the writer examined 26 opossums. The animals were collected over a period of a year and a half from four widely separated areas within the state of Georgia (Fort Valley, 6; Dublin, 2; Fort Stewart, U.S. Army Reservation, 17; Perry, 1). Fifteen genera comprising about 17 species of helminths were recovered from hosts collected. This material consisted of about 11 species of nematodes (including 1 described herein as new), 3 of trematodes, 1 of cestode, and 2 of Acanthocephala. Several of the parasites collected are of particular significance either because of their pathogenicity to the opossum or their infrequent occurrence in this host-species. Brief discussions of these worms are included. Measurements are reported in millimeters unless otherwise stated.

NEMATODA

Genus *Capillaria* Zeder, 1800

Capillaria longicauda Freitas and Lent, 1935

This nematode, identified by Mrs. MayBelle H. Chitwood, was collected from the esophagus of 3 opossums obtained from Fort Stewart. Infections ranged from 1 to 6 worms per animal. Read (1949 a, b) discussed and presented a key to the capillarids of North American mammals; however, of the 18 species cited, *Capillaria* from the opossum was not recorded. *C. auritae* Travassos, 1914, was originally described from *D. aurita* in Brazil. The Georgia material apparently is distinct from this species.

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Genus *Dipetalonema* Diesing, 1861
Dipetalonema sp.

Several filarioid nematodes were recovered from the lungs and esophagus of a single opossum taken at Fort Stewart. These specimens were turned over for study to Dr. Everett E. Wehr. In a communication received from him, the worms were identified as "*Dipetalonema* sp., probably *D.* n. sp." It is presumed that a complete description with species identification will be presented at a later date. It is interesting to note that another member of this group, *Dipetalonema pricei* (Vaz and Pereira, 1934), has been reported from subcutaneous tissue of the opossum.

Genus *Dirofilaria* Railliet and Henry, 1911
Dirofilaria sp.

A single specimen of this genus was obtained from the heart of an opossum from Fort Stewart. Species determination has not been attempted pending the collection of additional material. Although microfilaria from *D. virginiana* was recorded by several investigators, including Price (1954) and Robinson (1954), reports of an adult dirofilariid in marsupials are rare. *Dirofilaria skjabini* (Travassos, 1925) apparently is the only adult member of the genus reported from such hosts.

Genus *Gongylonema* Molin, 1857
Gongylonema longispiculum Schul'ts, 1927

Gongylonema longispiculum, also identified by Mrs. Chitwood, was collected from esophagi of 7 Fort Stewart opossums. The writer also has taken the species from an opossum in Louisiana. Infections ranged from 2 to 4 worms per animal. Several of the spirurids were so deeply embedded within the esophageal wall that it was impossible to remove them intact. *G. longispiculum* was originally described from *Citellus musicus* and *Spalax microphthalmus* by Schul'ts (1927). It has since been found in a number of species of "gophers," *Citellus* spp., but there is no record of its having been collected from the opossum in the Host Section of the Index Catalogue of Medical and Veterinary Zoology, Agricultural Research Center, Beltsville, Maryland.

The original description of *G. marsupialis* from the mucosal membrane of the esophagus of *D. aurita* as given by Vaz and Pereira (1934) was based upon 2 complete female specimens and the anterior end of another. The male of the species was described by Freitas and Lent (1937) as having 2 short, heavy spicules. Therefore, this species apparently is specifically distinct from *G. longispiculum* which has 1 spicule that is short (0.19 to 0.20) and another that is long (about 17.8).

Genus *Gnathostoma* Owen, 1836
Gnathostoma didelphis Chandler, 1932

As pointed out by Babero et al (1959), gnathostomes appear to be endemic in certain mammals of Georgia, having been collected from opossums (15), skunks (2), and raccoons (18). A North American record of cysts of larval gnathostomes in a snake (*Agkistrodon piscivorous*) was reported by Babero and Shepperson (1958). Certain worms which fitted the description of *G. didelphis*, as given by Chandler, were collected from the liver of opossums in each study area. The range of infection was 2 to 7 gnathostomes per host. Generally, such infections could be

immediately recognized by the presence of characteristic "gnathostome-scars" on liver surfaces. In several instances the worms were observed to extrude from the liver. Macroscopically, the organ appeared dark and cirrhotic. Histological sections of infected livers have not yet been made.

Gnathostoma sp.

From 1 to 11 specimens of gnathostomes were recovered from stomachs of 6 opossums. Although liver gnathostomes were collected from animals in each study area, stomach infections were observed only in animals obtained from Fort Stewart. In most cases, animals with stomach gnathostomes did not have them in the liver. In one instance, however, multiple infection was observed. Within the stomach the parasites were deeply embedded within gastric ulcers, each of which usually contained only 1 worm. Occasionally, both the head and tail regions of a worm were observed extending through the gastric mucosa from a tunnel within the muscularis. Complete perforation of the stomach wall was seen in 3 animals.

There appears to be some confusion concerning the identity of *Gnathostoma* Owens, 1836, occurring in the stomach of marsupials. *G. turgidum* Stossich, 1920, was reported from *D. virginiana* by Dikmans (1931) and from *D. aurita* and *D. azare* by Travassos (1925). The gnathostome identified by Dikmans later was synonymized with *G. didelphis* by Chandler, who suggested that the latter parasite may be co-specific with *G. horridum* (Leidy, 1856) from *Alligator mississippiensis*. *G. didelphis* from *Philander laniger pallidus* Thomas was reported by Foster (1939) and *G. spinigerum* Owen, 1836, from *D. marsupialis* by Caballero (1958). Because of certain apparent differences (body-size and cuticular spination) and the unusual pathogenicity established, the writer prefers not to assign the stomach gnathostomes of Georgia opossums to a definite species until the present study on morphologic variation by Dr. Caballero has been completed. In addition to the Georgian material, specimens recently collected from the stomach of Louisiana opossums will be included in this study.

Genus *Trichuris* Roederer, 1761

Whipworms are not commonly found in marsupials. Foster (1939) lists only 3 species, *T. peramealis* Baylis, 1932, *T. marsupialis* Foster, 1939, and *T. minuta* Rudolphi, 1819. *Trichuris urichi* and *T. reesali* were described by Wolfgang (1951). From *D. virginiana*, only *T. minuta* has been reported. *Trichuris* sp. listed by Dikmans probably is assignable to this species.

Trichuris minuta Rudolphi, 1819

This whipworm was recovered from the intestines of 13 opossums. The range of infection by the species was 6 to slightly over 200 specimens per animal. Although *T. minuta* (syn. *Trichocephalus minutus* Rud., 1819) appears to be somewhat endemic in occurrence, it has been widely reported from several species of opossum, including *"Didelyphys azare, D. cancrivorus, D. dom., D. nudicaudata, D. virginiana"* (See Stiles and Hassal, 1920).

Chandler (1930) pointed out the value of employing certain morphological characteristics, along with those previously used, in identifying species of the genus. He found that for a given species of *Trichuris* the lengths of the cloaca and its diverticulum, ejaculatory duct, and vas deferens were remarkably constant. The

redescription of the male of *T. minuta* as given by Chandler was based upon a single fragmentary specimen whose spicular sheath was not extruded. The writer has examined about 30 complete males and a more detailed description based upon the Georgia material and employing the characters used by Chandler is given.

Trichuris minuta Rudolphi, 1819
(Figs. 3, 4)

Diagnosis: (Male) Total length 17.8 to 25.8; esophageal region 11.2 to 15.1; thick portion of body 6.6 to 10.7. Width of head about 0.015; of mid-esophageal region 0.079 to 0.084; at junction of esophagus and intestine 0.15 to 0.18; or rear portion of body 0.25 to 0.29. Spicule 0.95 to 1.15 long; 0.018 to 0.021 wide at proximal end, 0.006 to 0.009 throughout most of length. Spicular sheath extending 0.030 to 0.138 beyond rear end of body and is usually cylindrical or sleeve-shaped, covered with small spines less than 1 micron long. Cloaca 1.72 to 1.82 long, thick and muscular. Spicular diverticulum 0.158 to 0.175 long, length may vary in accordance with action of strong retractor muscle at cephalic end. Ejaculatory duct straight 3.50 to 4.34 long. Vas deferens extending anteriad to about level of esophagus there turning and giving rise to testis, 2.14 to 3.09 long. Testis originating 0.92 to 1.80 from posterior end of body, without lobations.

Host: Didelphis virginiana
Location: Intestine
Locality: (Georgian material) Fort Stewart, Fort Valley, and Dublin.
Specimens: U. S. National Museum Helminthological Collection No. 39036.

Trichuris didelphis n. sp

An opossum obtained from the Perry locality harbored over 100 whipworms. When morphological studies of these specimens were made and compared with known members of the genus, it was concluded that these trichurids, although resembling several members of the genus reported from marsupials, could not justifiably be assigned to any of them. Therefore, the Perry material is described as new.

Trichuris didelphis n. sp.
(Figs. 1, 2, 5, 6)

Diagnosis: (Male) Total length 12.5 to 16.7; esophageal region 8.50 to 10.7; thick portion of body 4.0 to 5.9. Width of head 0.018 to 0.021; of mid-esophageal region 0.084 to 0.112; at junction of esophagus and intestine 0.140 to 0.182; of rear body 0.210 to 0.280. Spicule 0.90 to 1.22 long; 0.017 to 0.021 wide at proximal end, 0.007 to 0.009 throughout most of length. Spicular sheath extending 0.098 to 0.154 beyond rear end of body, globular, vase-, or pear-shaped in outline (figs. 5, 6) usually covered with small spines less than 1 micron in length. Cloaca 1.28 to 1.60 long, thick and muscular. Spicular diverticulum beginning posterior to middle of cloaca, leaving cloaca 0.336 to 0.390 from posterior end of body. Diverticulum 0.14 to 0.20 long. Ejaculatory duct straight, 2.24 to 2.91 long. Vas deferens 2.00 to 2.60 long, extending anteriad to level at or slightly below esophageal-intestinal junction there turning and giving rise to testis. Testis originating at a level 0.072 to 0.099 from posterior end of body.

(Female): Total length 13.3 to 20.1; esophageal region 8.5 to 13.7; thick portion of body 4.8 to 6.4. Width of head 0.012 to 0.015; of mid-esophageal region 0.099 to 0.102; bacillary band absent; maximum width of posterior portion of body 0.252 to 0.350. Muscular vulva non-salient and smooth, circular or oval in outline, 0.074 to 0.140 from intestinal-esophageal junction. Vagina (including slightly convoluted ovijector) 0.63 to 0.84 long. Ovary arising 0.182 to 0.322 from posterior end of body, running anteriorly to a level 0.55 to 0.77 from vulva there giving rise to oviduct. Oviduct taking a tortuous course posteriorly, turning then at a level 0.42 to 0.98 from anterior end giving rise to uterus. Uterus usually filled with eggs throughout most of length, with or without constrictions. Eggs 0.062 to 0.069 long by 0.030 to 0.031 wide, excluding opercula 0.045 to 0.054 long. Rectum 0.15 to 0.17 from subterminal anus.

Host: Didelphis virginiana
Habitat: Intestine
Locality: 5 miles east of Perry, Georgia
Type Specimens: U. S. National Museum Helminthological Collection No. 39037. (holotype male and allotype female); No. 39038. (paratype males and females).

When only the characteristics of genitalia of male specimens were considered, it was noted that, except for variations in length, *T. didelphis* showed close resemblance to most of the trichurids cited herein from marsupials (table I). However, when characteristics of female specimens were studied along with certain minor features (presence of a papilla, shape and spination of bursal sheath, length of worm) of males, the species could be distinguished.

Trichuris didelphis differs significantly from *T. peramealis.* In addition to being a much larger worm, it has a longer ejaculatory duct, cloaca and vas deferens, as well as a larger egg-size and a different-shaped spicular sheath. The sheath in *T. peramealis* is sleeved-shaped and spiny proximally for about one-half its length and its distal half is a smooth membranous expansion.

The new species differs from *T. minuta* in having a slightly smaller body-size and in its shorter cloaca and ejaculatory duct. It also differs in the absence of a bacillary band and in the shape of the spicular sheath.

Trichuris didelphis differs from *T. reesali* in several respects: (1) Its longer ejaculatory duct and vas deferens; (2) the shape of the spicular sheath (sleeve-shaped in *T. reesali*); (3) the absence of a bacillary band; (4) having eggs with protruding plugs; (5) the absence of a retractile papilla at the posterior end of the body; (6) its shorter vagina-ovijector complex; (7) having a non-salient vulva without a definite rim, and (8) in the posterior extension of the ovary.

By the size of the anterior and posterior body and by the shape of the spicular sheath, *T. didelphis* more closely resembles *T. marsupialis.* However, the new species may be readily separated from this species by its longer spicule and by most other male reproductive structures. It may be distinguished further by: (1) the absence of a bacillary band; (2) possessing a non-salient vulva without projections, and (3) its longer vagina-ovijector complex.

Through the courtesy of Drs. A. McIntosh and T. W. M. Cameron, the writer was permitted to examine some trichurids from marsupials. From Dr. McIntosh were obtained 2 groups of specimens—*T. marsupialis* (U. S. Nat. Mus. Helm. Col. Nos. 42990, type; 42991, paratype), from *Philander laniger pallidus,* and *Trichuris* sp. (No. 47762); deposited by Dr. C. P. Read from a Texas opossum (probably, *D. virginiana*). From Dr. Cameron were obtained 2 female and 1 male specimen of *T. reesali,* from *Didelphis marsupialis insularis.* The latter specimens comprised a portion of the material upon which Wolfgang based his original description of the species. Although the writer considers the Texas specimens to be co-specific with *T. minuta,* they did present some interesting features which could possibly cast some doubt concerning the identity of trichurids in North and Central American marsupials. When morphological comparisons were made with male specimens of the available groups of trichurids, the redescription of *T. minuta* by Chandler, and the description of *T. marsupialis* by Foster, and *T. urichi* and *T. reesali* by Wolfgang, it could be interpreted that certain of the differential characters of the genitalia employed by Chandler, within a species, did vary with the length of the worm (table I). It is highly probable that lengths of these characteristics may be constant for whipworms of the same size but may vary proportionately in worms of different sizes.

Except for the degree of papillated projections about the vulva and the length of the spicule tube, *T. urichi* shows a marked resemblance to *T. marsupialis.* The "blister-like protuberances" described by Wolfgang for *T. urichi* also were observed

TABLE I. *Comparison of approximate lengths in millimeters of certain differential characters* in seven groups of male trichurids.*

Species	TL	PB	ESOPH	SP	CL	EJAC	VD
T. minuta (Chandler—1 specimen)	29	9.8	20+	1.3	2.4	4.3	3.2
T. minuta (Babero—30 specimens)	17–25	7–11	11–15	0.9–1.2	1.7–1.8	3.5–4.3	2.0–3.0
Trichuris sp. (Read—6 specimens)	13–17	4–6	8–10	0.9–1.2	1.3–1.6	2.2–2.9	2.2–2.6
T. didelphis (Babero—30 specimens)	13–17	4–6	8–10	0.9–1.2	1.3–1.6	2.2–2.9	2.2–2.9
T. recsali (Wolfgang—4 specimens)	12–16	4–6	8–10	0.9–1.1	1.4	1.8–2.5	0.9–1.6
T. urichi (Wolfgang—"several specimens")	12–15	4.6	8–9	0.8–0.9	0.5–0.7	1.5–2.6	0.8–1.6
T. marsupialis (Foster—2 specimens)	12–15**	4–5	8–10**	0.7	0.9	1.6	1.4

* TL, total length; PB, posterior body; ESOPH, esophagus; SP, spicule; CL, cloaca; EJAC, ejaculatory duct; VD, vas deferens.

** Range of TL and ESOPH were obtained by multiplying the figures given for PB by 2, since Foster stated that ESOPH was not more than twice the length of PB.

in the male specimen of *T. marsupialis* examined by the writer. Although certain plaques of the esophageal area have been described for some trichurids, those observed in *T. marsupialis* and figured for *T. urichi* possibly were due to the delay or manner of fixation of the worms. Similar inflations were frequently observed by the writer in some specimens of both *T. didelphis* and *T. minuta*, but not all. It was pointed out earlier (see description of *T. minuta*) that the length of the spicular tube may vary with the action of the retractor muscle. It is therefore suggested that *T. urichi* be considered a synonym of *T. marsupialis*.

The variability of the prepuce-like expansion surrounding the spicule of trichurids was pointed out by several investigators, including Schwartz (1926). The present writer has also observed some variation of this structure. On the other hand, for the Georgia and Texas specimens of *T. minuta* and those of *T. didelphis* the shape of the spicular sheath did not vary to such an extent that these 2 species could not be immediately distinguished (figs. 3, 4, 5, 6). The shape and armature of the spicular sheath, therefore, when considered along with other morphological features of the worm may be reliable characters in species differentiation.

Other Nematoda

In addition to the nematodes discussed, 3 other members of this group were collected from opossums. The worms recovered and the number of hosts infected are as follows: *Cruzia americana* Maplestone, 1930 (22); *Physaloptera turgida* Rudolphi, 1819 (23); *Viannaia bursobscura* Dikmans, 1932 (16).

CESTODA

Genus *Mesocestoides* Vaillant, 1863
Mesocestoides variabilis Mueller, 1927

Mesocestoides variabilis represents the only cestode recovered from hosts during this survey. The parasite was collected 6 times, as follows: Fort Valley (2); Fort Stewart (3); Dublin (1). The range of infection by the worm was 1 to 4 per host.

TREMATODA

Flukes were occasionally encountered in opossums of Georgia. The ones recovered are *Brachylaime virginianus* (Krull, 1934)* (4), *Diplostomum variabile* Chandler, 1932 (6), and *Rhopalias macracanthus* Chandler, 1934, (4). The former trematode was collected from opossums at Fort Valley (2) and Fort Stewart (2); the range of infection was 2 to 12 worms per animal. The latter parasites were recovered from Fort Stewart opossums and the range of infections were 5 to 28 and 2 to 6, respectively.

ACANTHOCEPHALA

Genus *Hamanniella* Travassos, 1915
Hamanniella tortuosa (Leidy, 1850)

Acanthocephalan infections of marsupials appear to be uncommon in occurrence. According to Van Cleave (1953), only 3 species have been reported from opossums: *H. tortuosa, H. microcephala* (Rudolphi, 1819), and *H. tumida* Van Cleave, 1947. In North America, only *H. tortuosa* has been reported, having been cited from Florida, Oklahoma, Texas, and Illinois. The organism probably is more widespread in its distribution, however, since the writer has collected it in Georgia and Louisiana. The extent of infections were observed to range from 2 to 20 worms per animal. The hemorrhagic lesions accompanying infections as described by the writer (1957) were not observed in hosts from the latter localities.

Unidentified Acanthocephala

Aside from the gigantorhynchids recovered from Georgia opossums, 2 animals from the Fort Stewart area were parasitized by a small number of Acanthocephala of an unknown genus. These specimens were turned over for study to Dr. D. R. Lincicome, Howard University, who will probably include their identification in a later publication.

SUMMARY

Twenty-six opossums were examined for helminth parasites. Fifteen genera comprising about 17 species were recovered. For the most part, the helminths reported herein appear to parallel those listed by other investigators. However, *Capillaria longicauda, Gongylonema longispiculum, Dipetalonema* sp., *Dirofilaria* sp., and an unidentified Acanthocephala apparently were reported for the first time from this host. Doubt has been expressed concerning the identity of the *Gnathostoma* occurring in the stomach of opossums. A description of *Trichuris didelphis* n. sp. and a redescription of *T. minuta* were given. *T. urichi* was considered a synonym of *T. marsupialis*.

ACKNOWLEDGMENTS

The writer is indebted to the following persons for assistance rendered during this study: the personnel of the 714th Preventive Medical Company, Fort Stewart,

* This nomenclature is in accordance with the orthography of the genus as presented by Kruidenier and Gallichio (1959, Tr. Am. Micr. Soc. **78:** 428–441). There appears to be some confusion as to the authority for the species (Ulmer, 1952, Iowa St. Col. J. Sci. **27:** 91–103; Sandars, 1957, J. Helm. **31:** 265–272). The authority cited may not be correct. The writer concurs with Byrd et al (1942, J. Tenn. Acad. Sci. **17:** 130–142) in that *B. opisthotrias* is specifically distinct.

Georgia, for aid in obtaining animals; Dr. Everett E. Wehr and Mrs. MayBelle H. Chitwood, Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Maryland, for their identification of certain nematodes; Dr. Allen McIntosh, also of the Animal Disease Laboratory, and Dr. T. W. M. Cameron, Institute of Parasitology, MacDonald College, Quebec, Canada, for making specimens of trichurids available for study. Special acknowledgment is extended to Miss Jacqueline R. Shepperson, Fort Valley State College, Georgia, for laboratory assistance rendered.

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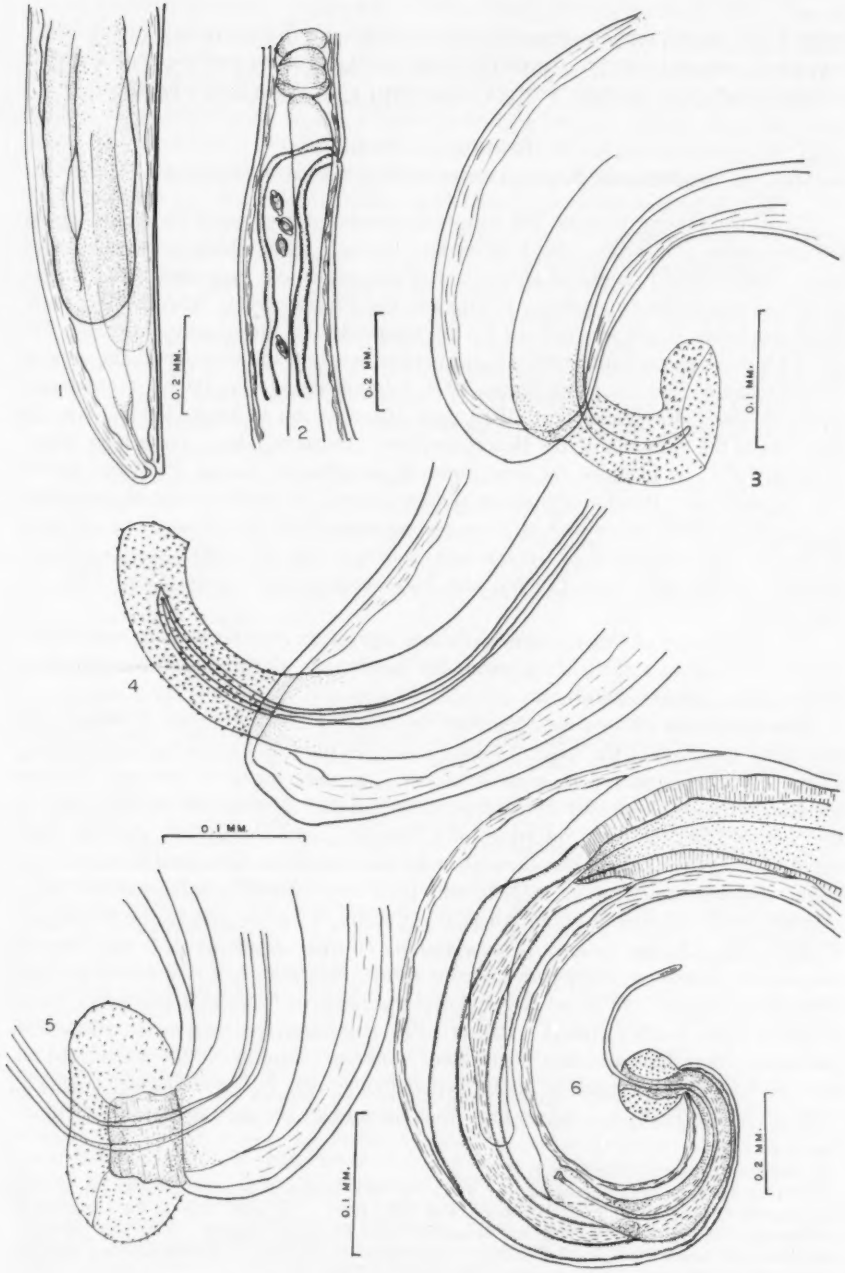
Explanation of Plate I

Figure 1. *T. didelphis,* n. sp., caudal end of female.
Figure 2. *T. didelphis,* n. sp., vulvular area of female.
Figure 3. *Trichuris* sp. (Texas), caudal end of male.
Figure 4. *T. minuta* (Georgia), caudal end of male.
Figure 5. *T. didelphis,* n. sp., spicular sheath of male.
Figure 6. *T. didelphis,* n. sp., caudal end of male.

PLATE I



THE LIFE HISTORY OF *BRACHYCOELIUM OBESUM* NICOLL, 1914, WITH A DISCUSSION OF THE SYSTEMATIC STATUS OF THE TREMATODE FAMILY BRACHYCOELLIDAE JOHNSTON 1912*

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Up to the present time no life history is known of any members of the genus *Brachycoelium* (Dujardin, 1845) Stiles and Hassall, 1898, which includes 10 species. Cheng (1958) published an account of this genus and suggested that the systematic status of *Brachycoelium,* whether in the Plagiorchiidae, Dicrocoeliidae, or Brachycoeliidae, might be resolved by the knowledge of life history patterns. He based his decision on adult morphological characteristics and considered the genera *Brachycoelium, Cymatocarpus* Braun, 1901, *Leptophallus* Lühe, 1909, *Glypthelmins* Stafford, 1905, *Margeana* Cort, 1919, and *Mesocoelium* Odhner, 1911, all to be members of the same taxon, the Brachycoeliinae (Dicrocoeliidae). In a later paper (Cheng, 1959) he included the new genus *Reynoldstrema* Cheng, 1959, but stated that the subfamily Brachycoeliinae should be elevated to familial rank as suggested by Johnston (1912) since such an arrangement seems to be the natural one as based in the life history patterns of *Glypthelmins quieta* (Stafford, 1900), as reported by Rankin (1944) and Leigh (1946), and *Mesocoelium brevicaecum* Ochi, 1930, as reported by Ochi (1930).

The life history of *Brachycoelium obesum* represents the first to have been determined in the genus and the third within the family. In all three cases the parasites utilize only 1 intermediate host.

Brachycoelium obesum was described by Nicoll (1914) from the intestine of a moribund specimen of the snake, *Contia aestiva,* in the London Zoological Gardens. Cheng (1958) considered *B. ovale* Byrd, 1937, a synonym of *B. obesum.* During the summer and fall of 1956 the author identified over 300 worms as belonging to this species from the small intestines of *Plethodon glutinosus* and *P. cinereus* captured at Mountain Lake, Giles County, and Charlottesville, Albamarle County, Virginia. During the summers of 1957 and 1958 over 50 additional specimens were obtained from the same hosts in Albamarle County, Virginia, and in the vicinity of Phoenixville, Chester County, Pennsylvania. Earlier Byrd (1937) reported *B. obesum* in *Desmognathus f. fuscus* from Rome, Georgia; in *Leiolopisma laterale* from New Orleans and Pearl River, Louisiana; and in *Plethodon glutinosus* from Calhoun Falls, South Carolina. Parker (1941) reported this parasite in *Plethodon glutinosus* from Reelfoot Lake, Tennessee. Although Rankin (1938) considered all the species of *Brachycoelium* to be synonymous with *B. salamandrae* (Frölich, 1789), Cheng (1958) has shown that 10 of the species are morphologically distinct.

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The systematic status of the subfamily Brachycoeliinae Looss, 1899, has been a long disputed question. Fuhrmann (1928), Byrd (1937), Parker (1941), Dawes (1946), and Patten (1952) all considered Brachycoeliinae a subfamily subordinate to the Dicrocoeliidae. On the other hand, Travassos (1926 and later), Lucker (1931), Mehra (1931 a,b) and Olsen (1937) each considered certain member genera to be subordinate to the Plagiorchiidae. Johnston (1912), however, proposed that the Brachycoeliidae should be considered an independent family. Morphologically, the members of the brachycoeline group reveal affinities to both the Dicrocoeliidae and the Plagiorchiidae; undoubtedly they are related families. Although Rankin (1938) reported the life history of *Glypthelmins quieta,* this information was insufficient to resolve the familial designation of Brachycoeliinae since the genus *Glypthelmins* was considered by Olsen (1937) and others not to be within the same subfamily as *Brachycoelium.* Ochi's (1930) description of the life-cycle of *Mesocoelium brevicaecum,* again, was only a partial solution since Dollfus (1933) and others considered the genus *Mesocoelium* as the monotypic member of the independent family Mesocoeliidae Dollfus, 1933. It became apparent that the solution of this puzzle rested with an understanding of the life history pattern within the type genus, *Brachycoelium.*

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MATERIALS AND METHODS

Because of the great similarity among the species of *Brachycoelium,* a concentrated study was initiated to determine consistent characteristics by which the species can be distinguished from one another. By studying over 350 adult specimens, it was determined that *B. obesum* can be separated from the others particularly by its large cirrus pouch which is approximately twice the diameter of the acetabulum in length.

Adult flukes removed from the small intestines of *Plethodon glutinosus* and *P. cinereus* from the proximity of Mountain Lake Biological Station, and from *P. cinereus* from around Charlottesville, Virginia, possessed embryonated eggs. Examinations of the feces of infected salamanders revealed many trematode eggs identical to those found in the uteri of the worms.

During a survey of helminths parasitic in various animals in the Mountain Lake area, the common land snail, *Zonatoides ligerus,* and the common slug, *Agriolimax agrestris,* were suspected as the intermediate hosts since both were found to be infected with a morphologically identical short-tailed xiphidiocercaria. The appearance of this cercaria strongly suggested that it might be the larval form of *Brachycoelium* sp. since it possesses short intestinal ceca and a flame-cell pattern of the 2 [(3+3+3)+(3+3+3)] type. Uninfected snails of the same species were found in the area of Duke's Pond, Charlottesville, Virginia. These were collected and used during the infection experiments.

Eggs collected from the feces of infected *Plethodon glutinosus* and *P. cinereus* were fed to these uninfected molluscs by placing them on moist oatmeal flakes which were readily ingested by pre-starved snails. The molluscs were examined at 30-minute intervals after feeding.

In addition to feeding eggs to *Zonatoides ligerus,* attempts were made to hatch them in vitro in strained pond water at room temperature (about 37C.) and at 50C.; in tap-water at room temperature and at 50C.; in amphibian physiological saline (0.65 percent NaCl) at room temperature and at 50C.; and in 0.5 percent formalin at 30C. Observations were made periodically up to 36 hours after the eggs were placed in the experimental culture media.

Fourteen specimens of *Triturus v. vididescens,* from a pond (Duke's Pond) where the newts were known not to be infected with intestinal trematodes, were employed as experimental definitive hosts. Examinations for eggs in fecal droppings of these newts by use of the zinc-sulfate technic reaffirmed the absence of intestinal flukes. Four to 7 metacercariae recovered

from experimentally and naturally infected snails were fed to 5 newts with the aid of a pipette. The metacercariae were placed in amphibian physiological saline. The long pipette was carefully inserted into the esophagus and the larvae were ejected into its lower portion. The hosts were not subsequently fed. Five additional newts were kept in individual dishes with little distilled water but no food and thus maintained for 7 days after which time 2 were fed 6 and 5 metacercariae respectively by the pipette method. The other 3 newts were each fed a piece of snail tissue from the proximity of the hepatopancreas known to include metacercariae. Each portion included approximately 15 to 20 metacercariae. The remaining 4 newts were maintained on a raw meat diet without attempting to infect them with metacercariae.

OBSERVATIONS

In every case, eggs observed in the terminal part of the worms' uteri contained immobile embryos suggesting that the activation of the miracidia is not brought about until the eggs come in contact with the digestive juices of the molluscan host. Further evidence of this is presented below.

Examination of the experimentally infected snails revealed that the time required for the activation and the hatching of the miracidium was found not to be consistent; however, the activation is dependent upon the ingestion of the egg by the mollusc and its coming in contact with the host's digestive juices. In 1 snail, eggs in the intestine, examined 30 minutes after ingestion, were not observed to be active; in 4 other snails the miracidia were observed to be somewhat active; in still others, eggs contained motile, fully developed miracidia in 30 minutes. These inconsistencies in the rapidity of initiating complete motility of the miracidium are perhaps due to the condition of the host. The physiological processes involved in the hatching of the egg within the intermediate host is unknown.

One snail was dissected 30 minutes after the ingestion of eggs from the feces of infected salamanders, but the eggs were allowed to remain in the exposed gut where they were continuously observed. The time of the commencement of motility of the miracidia varied; however, most of them were active after one and a half hours. Escaping miracidia were also seen during this period.

The attempts at hatching the eggs in vitro were not successful. No symptoms of motility of the embryos were observed. Plasmolysis occurred in the eggs placed in the 0.5 percent formalin solution after 5 hours. On the basis of these results it would seem improbable that free-living miracidia hatch from the eggs in nature.

First and second generation sporocysts, cercariae, and metacercariae were recovered from the hepatopancreas of experimentally infected *Zonatoids ligerus* and *Agriolimax agrestris* from 13 to 20 days after ingestion of the eggs.

The first 5 newts, which were fed metacercariae by the pipette method, were examined at intervals ranging from 2 to 11 days and were found to be free of intestinal helminths. Four days after the experimental infection of the pre-starved newts, 4 of them were examined, and 2 revealed 3 and 2 young adults, respectively, attached to the epithelial lining of the small intestine. Fourteen days after the introduction of the metacercariae, the last newt was examined and 3 young adults were recovered from the gut. In each of the cases where young adults were recovered, the metacercariae had been introduced with a piece of the molluscan tissue.

The 4 uninfected newts were examined in 14 days and were all found to be free of helminth parasites.

STAGES IN THE LIFE CYCLE

The young adults were fixed in Carnoy's (6:1:1), stained with Harris' alum

haematoxylin. The cercariae and metacercariae were fixed and stained in the same manner. The following descriptions of the egg, miracidium, and first and second generation sporocysts are based on the study of living specimens with a phase-contrast microscope since attempts to fix and stain these were unsuccessful. All measurements are given in millimeters.

The Egg (Figs. 1 and 2)

All of the mature specimens of *Brachycoelium obesum* recovered from the salamander hosts possessed eggs in their uteri. The operculated eggs situated in the descending limb of the uterus are yellowish-white and when examined under the oil immersion objective are observed to contain the early embryo and yolk material. Those eggs situated in the terminal portion of the uterus are dark brownish-yellow and show a distinct point at the adopercular end. These enclose a fairly definitive miracidial form. These eggs measure 0.034 to 0.052 by 0.024 to 0.036.

From the evidences presented under observations, it is strongly suspected that although embryonic development does occur within the egg while still within the parent, the complete activation of the miracidium is not attained until the egg reaches the molluscan host. Free-living miracidia do not appear likely since attempts to hatch eggs in vitro in various media proved unsuccessful.

The Miracidium (Figs. 6–11)

The following description of the miracidium of *Brachycoelium obesum* is based on 15 individuals as seen in the alimentary tract of *Zonatoides ligerus* between 30 and 180 minutes after ingestion of the eggs.

Description: Body oval, slightly broader at anterior end, 0.044 to 0.045 long, 0.028 to 0.032 wide; prominent apical papilla; entire body surface covered with cilia; penetration glands, 2 in number, situated at midlength or slightly anterior to midlength of body, each measuring 0.007 to 0.008 by 0.004 to 0.005; germ balls situated in posterior half of body; in one specimen (fig. 11) a poorly developed sausage-shaped body (sporocyst?) seen in the posterior region of the body.

The Primary Sporocyst (Fig. 3)

The following description of the first generation or mother sporocyst is based on 5 specimens which were removed from the hepatopancreas of *Zonatoides ligerus* by teasing away the host's tissue and examining them in the living state with a phase-contrast microscope. This proved to be the most effective method since stained sporocysts in in toto mounts usually ruptured because of their extremelv thin walls and great sensitivity to changes in osmotic pressure. Although primary sporocysts were recovered from the molluscan hosts 13 to 20 days after the ingestion of eggs, it is suspected that it did not take 13 or more days before the appearance of primary sporocysts since on the 13th day secondary sporocysts and a few cercariae were also seen.

Description: Opaquely white, elongate, amorphous, unbranched sacs, 0.52 to 0.57 long (average 0.55) and 0.175 to 0.2 wide (average 0.185); sporocyst wall semi-transparent; no birth pore observed, suggesting that the escape of the secondary sporocysts is accomplished through the rupture of the wall; distinct germ balls of varying sizes, 0.02 to 0.04 in saccular cavity, more numerous towards one end; young secondary sporocysts, 0.09 to 0.25 long, occupying the entire saccular cavity.

The Secondary Sporocysts (Figs. 4 and 5)

The secondary or daughter sporocysts are also found in the hepatopancreas of

the gastropod hosts and can be distinguished from the primary sporocysts by their greater length and width, the cercariae contained within, and the presence of a distinct anterior end. The following description is based on 6 specimens examined in the living state with phase-contrast.

Description: White, opaque, elongate sacs with anterior end more pointed; body 1.4 to 1.62 long, 0.24 to 0.37 wide; germ balls of varying sizes, 0.029 to 0.145 in greatest diameter, in posterior region of body; sporocyst wall composed of single layer, not of 2 layers as described by Denton (1944) in *Eurytrema procyonis* Denton, 1942; saccular cavity enclosing cercariae in varying stages of development, those situated towards anterior end more fully developed; no birth pore observed, suggesting the rupture of thin body wall to permit escape of mature cercariae.

The Cercaria (Figs. 12, 13, 14)

Mature cercariae escaping from the secondary sporocysts are found in the hepatopancreas and actively migrating from it. The descriptions of the cercaria of *B. obesum* is based on 10 individuals taken from the periphery of the hepatopancreatic tissue. These were fixed in Carnoy's (6:1:1) and stained with Harris' alum haematoxylin.

Description: Xiphidiocercaria; body elongate, tail broad and short; entire body 0.168 to 0.237 (average 0.194) long, 0.061 to 0.077 (average 0.064) wide; cuticle spinous towards anterior end of body; anterior sucker 0.041 to 0.048 (average 0.045) by 0.037 to 0.048 (average 0.041); prepharynx short, invisible in some; pharynx large, muscular, 0.016 to 0.022 (average 0.019) in diameter; esophagus short, inconspicuous in some, not more than 0.002 long; intestinal ceca short, 0.028 long, not reaching level of acetabulum; group of 3 penetration glands on each side of pharynx; acetabulum 0.024 to 0.041 (average 0.035) by 0.034 to 0.044 (average 0.037), situated in posterior half of body. In apparently fully developed cercariae, genital primordium, irregularly shaped, midway between dorsal and ventral surfaces, posterior to acetabulum, 0.013 to 0.034 by 0.013 to 0.02; anlagen of testes, seminal receptacle, uterus, cirrus pouch and vitelline glands not apparent; excretory pore medioventral, at posterior terminal of body proper, although in younger specimens the definition of the body proper is not clearly demarked, however, in older specimens a delimiting constriction is present in area of pore. Excretory vesicle of modified Y-type with long stem and inconspicuous cornua branching dorsal to genital primordium; flame-cell formula 3 [(3+3+3) + (3+3+3)], determined from living specimens.

In the body cavity of the gastropod host these cercariae lose what is the true tail, i.e. that portion of the posteriorly projecting appendage posterior to the excretory pore. The body then tends to straighten out resulting in an elongate, non-encysted, oval metacercaria.

The Metacercaria (Figs. 15, 16)

The following description of the metacercaria is based on 6 of the most advanced forms which were recovered from the body cavity of *Zonatoides ligerus.* These were fixed in Carnoy's and stained with Harris' alum haematoxylin.

Description: Body elongate, oval, with cuticle spinous in anterior portion, 0.168 to 0.215 (average 0.179) long, 0.061 to 0.07 (average 0.063) wide; oral sucker 0.044 to 0.51 (average 0.048) by 0.041 to 0.051 (average 0.046); prepharynx inconspicuous, leading into pharynx; pharynx 0.01 to 0.015 (average 0.013) in diameter; esophagus characteristically short, 0.002 in length; intestinal ceca short, reaching anterolateral margin of acetabulum; penetration glands as described in cercaria; acetabulum posterior to middle of body, 0.034 to 0.041 (average 0.036) by 0.037 to 0.048 (average 0.043); genital primordium immediately posterior to acetabulum in older specimens (fig. 16) and more posteriorly in younger ones (fig. 15). In older specimens primordia of testes appearing submedially, posterior to genital primordium, testes obliquely tandem. Other portions of male and female reproductive systems not observed. Excretory pore terminal, leading into long stem of Y-shaped excretory vesicle; flame-cell formula 2 [(3+3+3) + (3+3+3)] as determined from living specimens.

The Adult (Figs. 18, 19, 20)

As described under materials and methods, the non-encysted metacercariae were

fed to uninfected *Triturus v. viridescens* and young adults were recovered in 4 to 14 days after feeding. The following description of the young adult is based on 4 specimens recovered from experimentally infected hosts. Two additional young adults were examined in the living state. Neither revealed active spermatozoa nor eggs.

Description: Body 0.321 to 0.428 (average 0.357) long, 0.168 to 0.245 (average 0.09) by 0.075 to 0.108 (average 0.086); prepharynx not observed; pharynx averaging 0.039 in diameter; esophagus 0.006 long; intestinal ceca short, bulbous, reaching level of acetabulum; acetabulum at midlength of body or slightly anterior thereto, 0.051 to 0.068 (average 0.059) by 0.041 to 0.065 (average 0.053); ovary round to oval, averaging 0.04 by 0.033, situated below tip of right testis averaging 0.062 by 0.052, left testis 0.058 by 0.045; cirrus sac large, 0.6 long, 0.2 wide, overlapping acetabulum dorsally; vitellaria not fully developed, 8 to 20 independent follicles on each side or oral sucker; excretory pore terminal; excretory vesicle of modified Y-type with long stem.

The description of the sexually mature adult is given by Nicoll (1914) and revised by Cheng (1958) to include the measurements of the various subsequent workers.

DISCUSSION

The description of the life history of *Brachycoelium obesum* sheds additional light on the long disputed question as to whether the Brachycoeliinae Looss, 1899, should be assigned to the Dicrocoeliidae, to the Plagiorchiidae Lühe, 1901, emend. Ward, 1917, or be considered of familial rank as proposed by Johnston (1912). All previous workers have based their opinions strictly on morphological studies of adult worms and all have conceded that the eventual solution of this question rests in the knowledge of the life-cycle patterns of the genera therein.

Rankin (1944) described the life cycle of *Glypthelmins quieta* (Stafford, 1900), a parasite of *Rana catesbeiana* and other species of *Rana*. He reported the existence of only one intermediate host, *Physa gyrina,* in which the trematode undergoes two sporocyst stages, the escaping cercariae encyst in the skin of *Rana* spp. and when the infected sloughed epithelium is ingested by the frog, the parasite establishes itself in the definitive host. Leigh (1946) reported the life-cycle of the same species but inferred that a certain degree of development occurs in the metacercaria while encysted in the frog's epithelium. It remained unresolved as to whether the frog serves as both a second intermediate and definitive host, or only as the definitive host with the encystment in its epithelium as merely a vehicle of transport not accompanied by development.

The question then arose as to whether *Glypthelmins* was correctly assigned to the Brachycoelinae. Since the life-cycle of no member of the type genus of the subfamily was known, and the position of *Glypthelmins* in that subfamily had been questioned by Travassos (1926), Lucker (1931), and Mehra (1931a); the description of the life history of that species, therefore, was not of immediate use in attempting to solve the systematic question.

Ochi (1930) reported the life history of *Mesocoelium brevivaecum* in which he described the presence of a short-tailed unencysted "adolescaria" in the only intermediate host, *Euhadra quaesita.* Yamaguti (1958, p. 400) erroneously recorded it as an encysted metacercarial form. The definitive host of this species is *Bufo vulgaris formosus* in nature. In addition to this host, he was able to infect experimentally *Rana nigromaculata, R. rugosa,* and *R. catesbeiana.* The larval stages of *M. brevicaecum,* like those of *Glypthelmins quieta,* are similar to those of *Brachycoelium obesum,* i.e. 2 sporocyst generations, cercaria, and metacercaria.

The pattern of larval generations, the morphology of the excretory vesicle (V to Y-shaped), and the general flame-cell formula of the 2 [(X+X+X)+(X+X+X)] type are of little value in determining the systematic status of the brachycoeline group since both the Plagiorchiidae and the Dicrocoeliidae reveal the same characteristics. The pattern of one intermediate host, however, separates the brachycoeline group from the other two families where in all known cases, the parasites apparently require 2 intermediate hosts. This difference strongly suggests the author's earlier contention (Cheng, 1958) that the genera *Brachycoelium, Glypthelmins,* and *Mesocoelium* are closely related. Furthermore, because of this marked distinction, the author proposes the adoption of Johnston's (1912) concept of raising Brachycoeliinae to familial rank, hence Brachycoeliidae.

Buttner (1951) reported progenetic metacercariae of *Brachycoelium salamandrae* encysted in the musculature of *Triton palamatus* and *T. alpestris* from Rennes, Ille-de-Vilaine, France. If the hypothesis that all members of the same trematode genus generally undergo similar life-cycles is valid, as has been repeatedly indicated, then the unusual condition, as reported by Buttner, must be interpreted as an accidental or alternative condition; however, the former interpretation appears more likely since *Brachycoelium obesum* is not known to exhibit any progenetic tendencies; even young adults found in the intestine of definitive hosts do not bear eggs.

The capability of the metacercariae of *Brachycoelium salamandrae* to encyst suggests that metacercarial cysts constituted an integral part of the life-cycle of an earlier progenitor of the modern members of the genus. The metacercaria of *Glypthelmins quieta* is known to require encystment in the skin of the definitive host. The parallel pattern of metacercarial behavior between the modern members of *Glypthelmins* and the progenitor of modern members of *Brachycoelium* further adds strength to the author's contention that the two genera are closely related. The parallel life history patterns of *Brachycoelium* and *Mesocoelium,* again, strengthens the contention that these two genera are also closely related.

In view of the information contributed by the life history of *Brachycoelium obesum,* it seems logical to consider the Brachycoeliidae, the Plagiorchiidae, and the Dicrocoeliidae as independent families subordinate to the superfamily Plagiorchioidea Dollfus, 1930.

LaRue (1957), in describing the cercariae of the members of the order Plagiorchiida LaRue, 1957, to which the Plagiorchioidea is subordinate, stated: ". . . encystment in invertebrates (chiefly arthropods), rarely in vertebrates." The short-tailed xiphidiocercariae of *Brachycoelium obesum* and *Mesocoelium brevicaecum* do not encyst, but are found in the gastropod host and the infection of the definitive host is brought about by the ingestion of infected gastropods. These observations warrant the modification of LaRue's description to read: ". . . encysted or non-encysted metacercariae in invertebrates, rarely in vertebrates."

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EXPLANATION OF PLATES

ZYG, zygote; VIT, vitelline membrane; EP, epidermal plate; AP, apical papilla; PG, primordia of penetration glands; RG, refractile granules; GB, germ ball; SP, developing sporocyst (?); EX, excretory pore; PT, primordia of testes; CER, cercaria; HEP, hepatic cells; MET, metacercaria.

PLATE I

FIGURE 1. Egg of *Brachycoelium obesum* from median section of uterus showing zygote and yolk cells.

FIGURE 2. Eggs of *B. obesum* from the ascending limb of the uterus in sexually mature adult.

FIGURE 3. First generation sporocyst enclosing second generation sporocysts and germ balls. Drawn from living specimens removed from hepatopancreas of intermediate host.

FIGURES 4 and 5. Second generation sporocysts enclosing cercariae and germ balls. Drawn from living specimens removed from hepatopancreas of intermediate host.

FIGURE 6. Egg of *B. obesum* recovered from feces of naturally infected *Plethodon cinereus.*

FIGURE 7. Egg of *B. obesum* enclosing miracidium recovered from feces of experimentally infected *Triturus viridescens.* The miracidium is non-motile.

FIGURE 8. Egg of *B. obesum* enclosing miracidium, recovered from small intestine of *Zonatoides ligerus* 40 minutes after ingestion. Miracidium motile.

FIGURE 9. Egg of *B. obesum* enclosing miracidium recovered from small intestine of *Zonatoides ligerus* 80 minutes after ingestion. Miracidium motile.

FIGURE 10. Miracidium of *B. obesum* recovered from small intestine of *Zonatoides ligerus* 90 minutes after ingestion of egg.

FIGURE 11. Miracidium of *B. obesum* recovered from small intestine of *Zonatoides ligerus* 2 hours after ingestion of egg.

PLATE II

FIGURE 12. Young cercaria of *B. obesum* recovered from daughter sporocyst. Camera lucida drawing of stained specimen.

FIGURE 13. Fully developed cercaria of *B. obesum* after escaping from daughter sporocyst. Camera lucida drawing of stained specimen.

FIGURE 14. Fully developed cercaria of *B. obesum* showing constriction at the body-tail junction at the level of the excretory pore. Camera lucida drawing of stained specimen.

FIGURES 15 and 16. Metacercaria of *B. obesum.* Camera lucida drawings of stained specimens.

FIGURE 17. Cross-section of *Agriolimax agrestris* through hepatopancreas at 10 microns, showing metacercaria on border of hepatic tissue. Camera lucida drawing.

FIGURES 18 to 20. Young adults of *B. obesum* drawn from stained specimens removed from small intestines of experimentally infected *Triturus viridescens.* Camera lucida drawings of stained specimens. Ventral view.

ANNOUNCEMENT

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES

(July 1960 to June 1961)

The Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Laboratory Branch, Atlanta 22, Georgia, announces the following schedule of laboratory refresher training courses with dates, names of courses, and duration of courses in weeks, as follows:

Oct. 10 to 21, 1960	Fundamentals of Virology (819)	2
Oct. 31 to Nov. 11, 1960	Laboratory Methods in the Diagnosis of Tuberculosis (855)	2
Nov. 28 to Dec. 2, 1960	Laboratory Methods in the Diagnosis of Rabies (826)	1
Dec. 5 to 9, 1960	Bacteriophage Typing of Staphylococci (856)	1
Jan. 9 to Feb. 3, 1961	Laboratory Methods in Medical Mycology (815)	4
Jan. 23 to Feb. 10, 1961	Serologic Methods in Microbiology (941)	3
Jan. 30 to Feb. 10, 1961	Laboratory Methods in the Diagnosis of Tuberculosis (855)	2
Feb. 13 to 24, 1961	Laboratory Methods in the Study of Pulmonary Mycoses (817)	2
Feb. 27 to Mar. 17, 1961	Laboratory Methods in Medical Bacteriology (838)	3
Mar. 6 to 10, 1961	Laboratory Diagnostic Methods in Veterinary Mycology (940)	1
Mar. 13 to 31, 1961	Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases (820)	3
Mar. 20 to 24, 1961	Special Problems in Medical Bacteriology (839)	1
Mar. 27 to Apr. 7, 1961	Laboratory Methods in Enteric Bacteriology (850)	2
Apr. 3 to 7, 1961	Laboratory Methods in the Diagnosis of Rabies (826)	1

The following courses are to be given by special arrangement only: Laboratory Methods in the Diagnosis of Malaria (805), 1 wk; Special Training in Virus Techniques (821), 2 to 4 wks; Typing of *Corynebacterium diphtheriae* (842), 1 wk; Special Problems in Enteric Bacteriology (851), 2 wks; Phage Typing of *Salmonella typhosa* (852), 1 wk; Laboratory Methods in the Diagnosis of Leptospirosis (853), 1 to 4 wks; Serologic Differentiation of Streptococci (854), 2 wks; Special Problems in Microbiology (942), 1 to 2 wks.

PLATE I

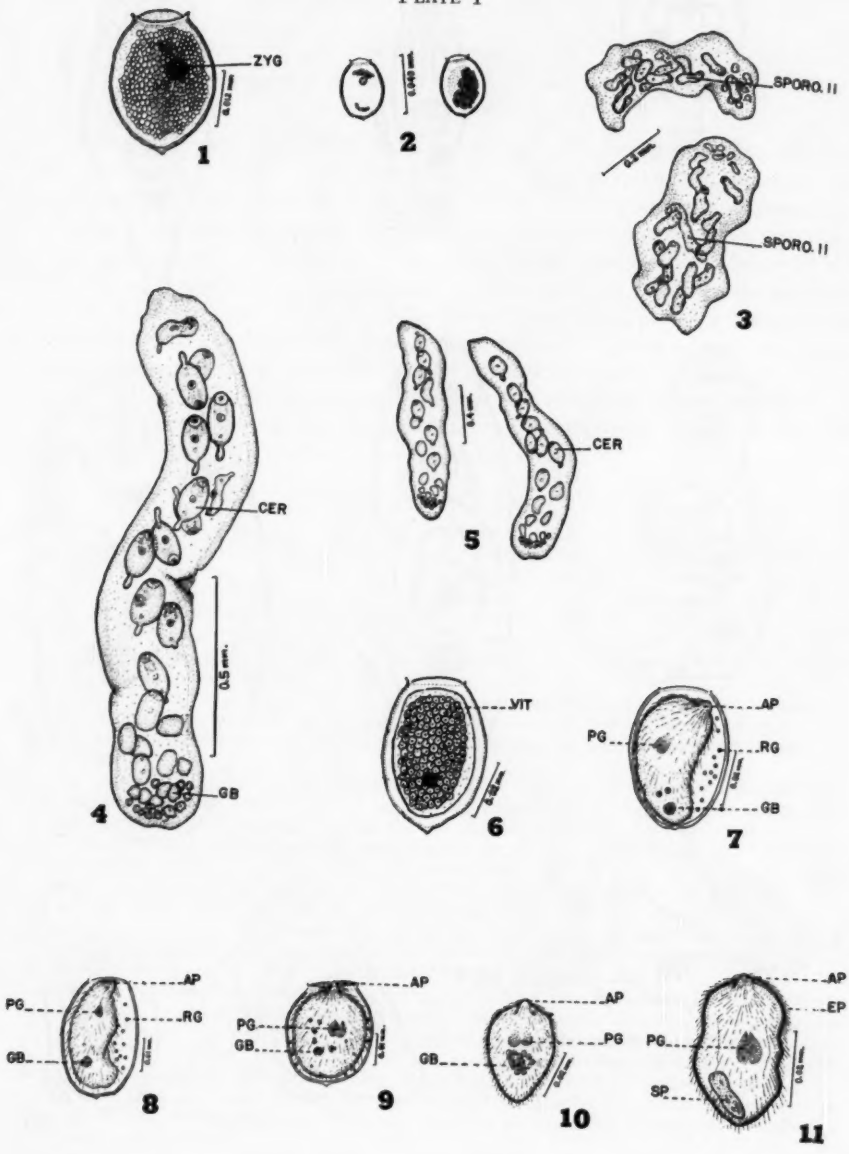
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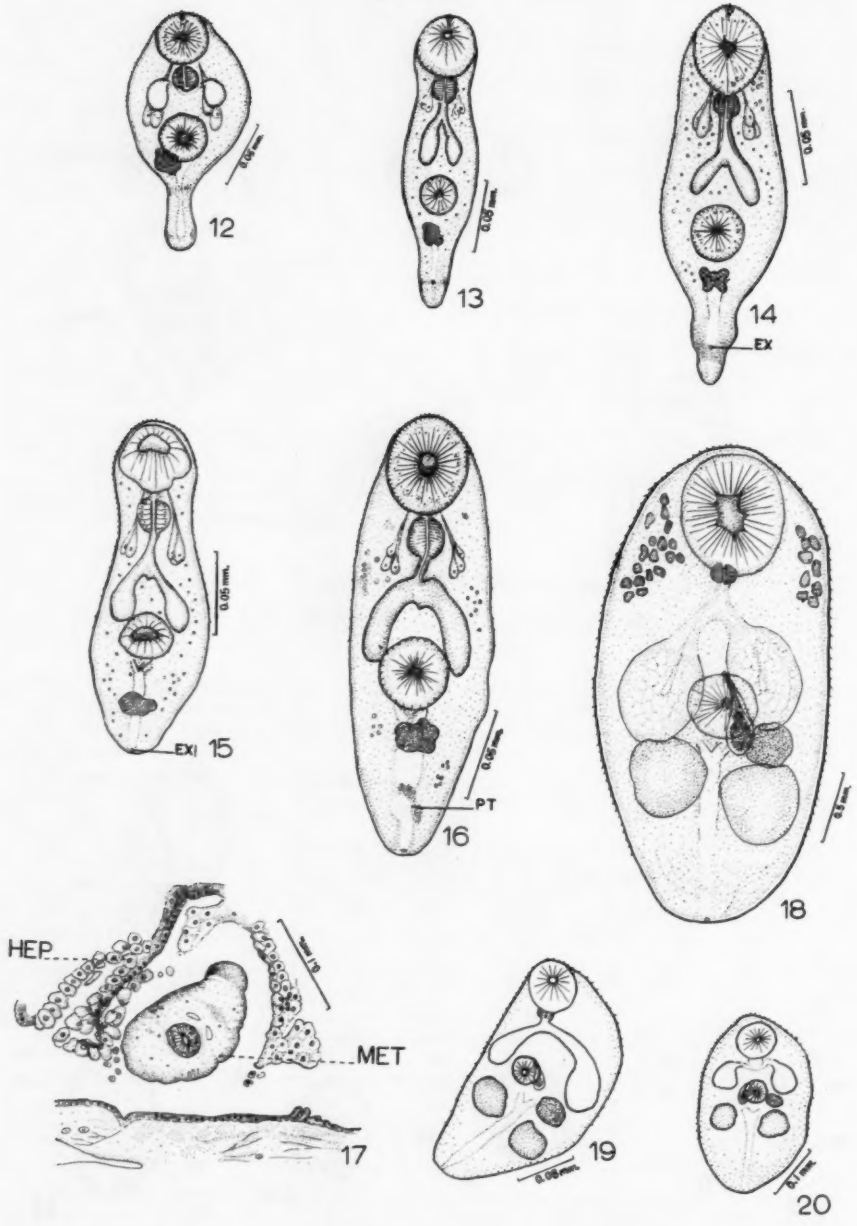
PLATE II



CHEMICAL NATURE OF CERCARIAL EYE-SPOT AND OTHER TISSUE PIGMENTS*

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The occurrence of pigmented eye-spots has been reported in the miracidial and cercarial stages of many digenetic trematodes. Several species of cercariae also have brown or black pigments in the body tissues occurring as granules, specks or strands. Although considerable information is available concerning the structure of eye-spots (Cort, 1915; Faust, 1917, 1918a, 1918b), the chemical nature of the pigments associated with them is not known. Faust (1918b) described the pigments located in the eye-spots of *Cercaria pellucida* as "melanoidin" which was considered to be a by-product of the nervous system. Apart from this casual account, no organized work seems to have been done to establish the chemical identity of these pigments. It is the purpose of this paper to report the results of various histochemical studies designed to reveal the chemical nature of the pigments associated with the eye-spots and the body tissues of three cercarial species.

MATERIALS AND METHODS

Three types of cercariae, *Euhaplorchis californiensis,* large pigmented echinostome and a schistosome were obtained from the marine snail, *Cerithidea californica,* found on the mud flats of Upper Newport Bay, California. Six other cercarial species, *Cloacitrema michiganensis, Parorchis acanthus,* Y-bladder cercaria, a large strigeid, a small strigeid, and a small echinostome also were included for various histochemical studies. Digestive glands and other tissues of the snail with single infection were removed, fixed, and sectioned at 5 to 10 microns in thickness. The solubility of the cercarial pigments was tested by immersing sections (8 to 10 microns thick) of formalin-fixed material in different solvents and examining them at intervals. The following solvents were used: Water, 1 percent cold sodium hydroxide, methanol, ethanol, 70 percent alcohol, acetone, chloroform, petroleum ether, carbon disulfide, xylol, benzene, ethyl ether, glacial acetic acid, concentrated hydrochloric acid, sulfuric acid, and cold and hot ethylene chlorhydrin.

In order to study the spectral properties, crude extracts of the pigments from the large pigmented echinostome cercariae in 0.2 percent hot sodium hydroxide were examined spectrophotometrically at wave lengths between 300 and 700 mμ.

The following histochemical tests were carried out for the cercarial pigments: Treatment with bleaching agents like hydrogen peroxide and chromic acids (Pearse, 1953); alkaline silver reduction methods of Montana-Masson (Pearse, 1953) and Bodian silver method (Dublin, 1943) for melanin; Schmorl reaction (Pearse, 1953) for lipofuscine, argentaffin granules, and melanins; Hueck's test (1912) for lipofuscine; PFAS reaction (performic acid Schiff Technique, Pearse, 1953) for lipochrome; diazo reaction (Pearse, 1953) for argentaffin granules; benzidine test for haemoglobin (Pearse, 1953); Gmelin test (Pearse, 1953) and Stein's test (1935) for bile pigments; Carr-Price test (Glick, 1949) for carotenoids; Perl's technique (Pearse, 1953) for iron; Prussian blue test (Glick, 1949) for organic iron; rubianic acid method of Okamoto and Utamura (Gomori, 1953) and methylene blue method of Mallory and Parker (1939) for copper and lead; Millon reaction (Pearse, 1953) for tyrosine; Romieu reaction (Glick, 1949) for tryptophane, and histochemical method of Becker et al (Pearse, 1953) for tyrosinase.

Efforts were also made to test the presence of tyrosinase in two types of cercariae, the large pigmented echinostome, and *Parorchis acanthus* by preparing extracts as described by Fox and Pantin (1941).

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Cercarial pigments were tested for flavones by studying their solubility in water, alcohol, and dilute acids and alkalis, and for haematin in alcoholic picric acid and alkalis.

To study the fate of pigmented eye-spots and other body pigments, cercariae of *Euhaplorchis*, were exposed to their second intermediate host the killifish, *Fundulus parvipinnis* in which the cercariae encyst on the brain. Metacercariae were recovered from the fish at intervals over a period of 6 weeks and studied histochemically.

MORPHOLOGICAL CONSIDERATIONS

The large pigmented echinostome cercaria develops within a redia which usually has a yellow or orange gut. This cercaria has 2 hemispherical eye-spots which are dorso-laterally placed at the level of the pharynx. A very conspicuous pigment spot medially located between the eye-spots gives the false impression of a third eye (fig. 4).

In the development of the cercarial eye-spot, a lens-like structure appears before pigment deposition (fig. 1). Pigment granules are laid down around the "lens" and gradually some of them become grouped in the form of a wide-mouthed cup (fig. 4). In more advanced cercariae heavy deposits of pigments appear over the central ganglionic mass dorsal to the pharynx. Three longitudinal pigment bands can also be noticed in fully developed cercariae (fig. 3). The 2 lateral bands of pigment seem to run parallel to the lateral nerve cords (fig. 6).

The sequence of development of these 3 pigment bands was studied in different stages of cercariae removed from rediae. Deposition of pigments along the 3 lines begins at the anterior end and gradually continues posteriad to reach the posterior end of the body in mature cercariae (figs. 1, 2 and 3). Pigment masses are also found concentrated in the vicinity of the oral sucker and many delicate lines of pigments radiate from the central pigment spot to the anterior end of the body (fig. 4). Under higher magnification and slight pressure, the pigment specks look like melanocytes with their dendritic extensions interconnected (fig. 5).

In cross sections of large pigmented echinostome cercaria, dark pigment granules could be found distributed at random in the hypodermal tissues, in addition to those of the three longitudinal pigment bands (fig. 7). However, there appears to be a concentration of pigment granules in the central region. No pigments could be detected either in the tail or in the cuticula of any of this species, even after alkaline silver reduction reactions.

The eye-spots which measure 0.02 by 0.012 mm, are connected with the central nervous system. In favorable sections it was possible to trace nerves going to the eye-cups from the central ganglionic mass. The eye-cups are composed of irregularly shaped, coarse brownish or black pigment granules.

Euhaplorchis californiensis, has a pair of compound eye-spots situated at about one-fourth the length of the body from the anterior end. In sagittal sections, they look like 2 cups set against each other and present a X-pattern (fig. 9). Each eye-spot looks spherical in whole mounts and measures 0.007 by 0.006 mm. The eye-cup consists of spherical pigment granules of uniform size measuring 0.7 micron in diameter. Nervous connections between the eye-spots and the central ganglionic mass were traced in favorable sections. Each compound eye seems to have 2 "lenses," one facing anteriad and the other posteriad.

Pigment granules are also found distributed at random in the hypodermal tissues in addition to those located in the eye-cups (fig. 12). There appears to be an increase in the number of pigment granules during the early metacercarial stage

(fig. 10). However, in the metacercariae recovered from the intermediate host, *Fundulus,* after 6 weeks, the quantity of pigment granules seemed to have considerably diminished (fig. 11). In old metacercariae, the eye-spots disintegrate, but remnants of them were still visible.

The schistosome cercaria has 2 large spherical eye-spots situated at about one-third the length of the body from the anterior end; each eye measures 0.01 by 0.01 mm. The eyes are cup-shaped and the pigment granules located in them are fairly large, spherical and grey or black in color. The granules measure 1 to 1.2 microns in diameter. The body tissues of schistosome cercariae are devoid of pigment granules.

CHEMICAL NATURE

The cercarial pigments were found to be insoluble in all the solvents used. Spectrophotometric examination of the crude extracts of the pigments from the large pigmented cercariae failed to show any definite absorption bands.

The pigments became decolorized in 10 percent hydrogen peroxide in about 24 to 40 hours and in chromic acids in about 10 hours.

The pigments were tested for the following substances with negative results: lipochrome, argentaffin granules, haemoglobin, haematin, bile pigments, carotenoids, flavones, lipofuscines, tryptophane, iron, copper, and lead. The tests, however, yielded positive results for melanin and tyrosine.

The result of the histochemical test for the detection of tyrosinase was not very conclusive. A positive reaction is indicated by darkening of the entire protoplasm of cells containing the enzyme. Slight blackening seemed to have taken place in the case of cercarial tissues; however, it was difficult to distinguish the pre-existing melanin granules from those formed by the reaction. Again, no darkening, which is supposed to indicate the presence of tyrosinase, was observed in any of the cercarial extracts. However, the extract of the large pigmented echinostome cercariae kept at 32C with or without air bubbling through it, turned brownish. This change in color is suggestive of the presence of the enzyme in this extract.

DISCUSSION

Various histochemical tests have shown that the cercarial pigments cannot be classified as tetrapyrrole pigments like haemoglobin, haematin, and bile pigments. Neither could they be considered as belonging to carotenoids nor flavones. The insolubility of the pigments in a variety of solvents, including several fixatives, has suggested their remarkable stability and chemical inertness. On the other hand, these pigments are sensitive to bleaching agents like hydrogen peroxide or chromic acids.

Cercarial pigments have been found to possess the properties of reducing ammoniacal silver solutions to black metallic silver and reducing ferricyanide to ferrocyanide. The former property is shared by melanins and argentaffin granules, and the latter by melanins, argentaffin granules, and lipofuscines (Pearse, 1953). However, the fact that cercarial pigments do not fall under the category of argentaffin granules is suggested by the negative diazo reactions.

Insolubility in organic solvents, positive Schmorl reactions, etc. are characteristics of both melanins and lipofuscines. However, lipofuscines do not have the property of reducing ammoniacal silver solutions, while cercarial pigments do

possess this property. Again, Hueck's test (1912), designed to differentiate lipofuscines from melanins, ruled out the possibility that cercarial pigments could be classified with lipofuscines. It has been found that cercarial pigments require less time for bleaching than do lipofuscines. Verne (1926) has reported the possible occurrence of small amounts of fixed iron in the dark lipofuscine granules recovered from the botryoidal tissues of the hirudinean worm. There was no indication of the presence of iron in the cercarial pigments.

All available evidence indicates that the pigments located in the eye-spots as well as in the body tissues of the large pigmented echinostome, *Euhaplorchis*, and schistosome cercariae belong to the indole group, namely melanin.

The occurrence of melanin in the eye-spots of cercariae is not surprising; pigment is a constant feature of photoreceptors. Both melanin and carotenoid pigments are known to be associated with the eyes of many invertebrates like the crustaceans and cephalopod molluscs. The orange-red stigma of green flagellates is composed of carotenoid pigments (Kylin, 1927). Carotenoid pigments like β-carotene and esterified astaxanthin have been reported to be present in the optic cushion of *Marthasterias glacialis* (Millott and Vevers, 1955).

Concerning the occurrence of melanin in the body tissues of the large pigmented echinostome and *Euhaplorchis* cercariae, it should be mentioned that this pigment is the most widely distributed of all pigments in animal tissues. Melanin is known to occur in the skin of turbellarians (Cloudsley-Thompson, 1946), in the ectoderm and endoderm of many coelenterates like *Metridium senile* (Fox and Pantin, 1941), in the integuments of several species of echinoderms (Verne, 1926), in the ink glands of cephalopods (Gessard, 1902), and in annelids like the leech, *Glossiphonia complanata* (Bradbury, 1957).

Faust (1918b), who studied the development of eye-spots in *Cercaria pellucida* and other species, stated that an ectodermal cup is formed in the vicinity of a nerve cell and pigment granules begin to appear only when the nerve ending occupies a position within the cup. This seems to suggest that the induction of pigment granules is a necessary consequence of appositional development of the nerve ending and the ectodermal cup. In the case of the large pigmented echinostome cercaria, it is the "lenses" that make their appearance first and pigment granules gather around them later. Here also the production of pigment granules appears to be the result of an inductive process.

Melanin in cercarial species studied, particularly in the large pigmented echinostome, seems to be closely associated with the nervous system. In this cercaria, the two lateral lines of pigments in the body tissues run parallel to the lateral nerve cords and the median pigment mass lies just above the "brain," the outline of which resembles that of the "brain" itself. It has been reported that in *Cercaria robusta* the nervous system is outlined superficially by the pattern and orientation of the pigment granules (Faust, 1918a). The neuro-epithelial origin of melanoblasts has been demonstrated by Becker (1942) and others. Faust (1918b) regarded "melanoidin" associated with the eye-spots of *Cercaria pellucida* and other species as by-products of the nervous system.

The presence of tyrosine, a precursor for melanin, in cercarial tissues has been indicated by the Millon reaction, though in variable quantities. The cystogenous gland cells of the large pigmented echinostome cercariae revealed very strong Millon reaction for tyrosine. Although the results of the tests for the demonstration of

tyrosinase were not very encouraging, there is an indication of its presence in the large pigmented echinostome cercariae. Cercarial tissues did not show any trace of copper, which has been reported to be an essential part of the tyrosinase prepared from mammalian, plant and insect tissues (Lerner and Fitzpatric, 1950).

An increase in pigment granules of the body tissues has been noticed in the case of metacercariae of *Euhaplorchis*, recovered from the killifish, *Fundulus*, during the initial period of infection. The rate of melanin formation, as the result of three basic substances, tyrosinase, tyrosine or other amino acid substrate and molecular oxygen is said to be controlled by several physico-chemical factors such as temperature, hydrogen ion concentration, oxidation-reduction potentials, etc. The production of increased amount of melanin in the larvae encysted in the fish host seems to be the result of a favorable operation of these various factors. Although the eye-spots break down in older metacercariae, pigment granules still persist. Remnants of the eye-spots are recognizable in adults of *Euhaplorchis* (Martin, 1950).

Pigmented eye-spots in miracidia and cercaria should be functional as photoreceptors like those of free-living invertebrates, during the brief period of their free-living existence. The wide-spread occurrence of eye-spots in cercarial and miracidial stages of the life-cycle of digenetic trematodes speaks for the evolution of these parasites from free-living ancestors. Degeneration of the eye-spots has occurred under conditions of endoparasitism (Faust, 1918b).

Wunder (1932) reported that the dark pigments of *Cercaria monostomi* serve to protect these larvae during encystment against injurious light rays. Strong pigmentation of the cercariae may be correlated with acute phototactic responses (Wesenberg-Lund, 1934). According to Kalmus (1941) melanin in the cuticle of insects may serve to provide increased toughness, protection from desiccation, injurious light rays, etc. How far these physiological functions could be realized by cercarial melanin is not known; however, it seems plausible that during the period of free-existence and encystment, cercariae are exposed to hazards of injurious light rays and desiccation and that melanin may be considered to offer a certain degree of protection.

Melanin pigments are formed as ultimate catabolic products of the amino acids tyrosine, tryptophane and phenylalanine. Melanin is excreted, as are other natural catabolites, sometimes by the skin and very often become incorporated in the materials of feathers, scales, etc. of birds. The hypoderm of brachyuran crustaceans contains a melanin-like pigment, which was experimentally shown to be an endogenous product of protein metabolism, and probably serves no particular metabolic function (Verne, 1920, 1923, 1926). Melanins found in the body tissues of the cercariae may be considered as products of protein metabolism with or without functional significance.

SUMMARY

Various histochemical tests have indicated that the brownish or black pigments located in the eye-spots and in the body tissues of 3 species of cercariae belong to the indole group, namely melanin. Tests for the detection of precursor system for melanin revealed that tyrosine is present in variable quantities in all the 9 species of the cercariae investigated. In 3 types of cercariae containing melanin pigments, tyrosine was found in greater concentration than in the non-pigmented types. Histochemical and other tests employed to detect tyrosinase yielded only inconclu-

sive results. Tests for copper and iron proved negative. A short discussion is given concerning the morphology, occurrence and possible role of melanin in the cercarial species. The fate of eye-spots in *Euhaplorchis* has been followed through the metacercarial stage recovered from the second intermediate host, *Fundulus*, over a period of 6 weeks.

ACKNOWLEDGMENT

The author is extremely thankful to Prof. W. E. Martin for his very helpful suggestions and criticisms during the course of this work.

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EXPLANATION OF PLATES

PLATE I

FIGURE 1. An immature large pigmented echinostome cercaria showing lenses of the eyes and pigments formed around them.

FIGURE 2. A large pigmented echinostome cercaria showing formation of pigment bands in anterior region.

FIGURE 3. Large pigmented echinostome cercaria showing 3 longitudinal pigment bands.

FIGURE 4. Large pigmented echinostome cercaria showing eye-spots and median pigment spot.

PLATE II

FIGURE 5. Large pigmented echinostome cercaria showing melanocyte-like pigment cells.

FIGURE 6. Transverse section of large pigmented echinostome cercaria passing through esophageal region.

FIGURE 7. T. S. of large pigmented echinostome cercaria showing general distribution of pigment granules in hypodermis.

FIGURE 8. Schistosome cercaria.

FIGURE 9. A sagittal section of *Euhaplorchis californiensis* showing compound or X-type eye-spots.

FIGURE 10. T. S. of *Euhaplorchis* (metacercaria) recovered from *Fundulus* after 15 hrs.

FIGURE 11. T. S. of *Euhaplorchis* (metacercaria) recovered from *Fundulus* after 6 weeks.

FIGURE 12. T. S. of *Euhaplorchis* (cercaria) showing distribution of pigment granules in the hypodermal tissues.

Abbreviations

BC, batonnet cells; CGC, cystogenous gland cells; CU, cuticula; E, esophagus; ES, eye-spots; HT, hypodermal tissues; L, lens; LNC, lateral nerve cord; LPB, longitudinal pigment band; MPS, median pigment spot; OS, oral sucker; PB, pigment band; PG, pigment granules; PH, pharynx; PS, pigment spots; VS, ventral sucker.

RESEARCH NOTE

A NOTE ON THE DISTRIBUTION OF THE PORCUPINE LOUSE *EUTRICHOPHILUS SETOSUS* (GIEBEL) (MALLOPHAGA).

A male porcupine *Erethizon dorsatum*, collected near Everett, Washington on September 27, 1957 by Mr. Loren Hansen, was examined for ectoparasites approximately 18 hours after its death. At this time numerous lice were observed crawling towards the apical ends of the hairs. These were collected and later identified as *Eutrichophilus setosus* (Giebel).

On June 1, 1959, a male and female porcupine were collected on Pine Mountain, Deschutes County, Oregon.

Lice were not observed on either animal at the time of collection, but 12 hours after death lice identified as *E. setosus* appeared on the hairs as in the previous case.

This louse has not previously been reported from Washington or Oregon. It has been reported from the following states: Alaska and California (Kellogg and Ferris, 1915, Stanford Univ. Publ., p. 59), Minnesota, Montana and Nebraska (Jellison, 1933, Tr. Am. Micr. Soc. **52**: 42–47), New Hampshire (Kilham, 1931, J. of Mammal. **12**: 318–319), New Mexico (Cockerell, 1902, Ent. Month. Mag. **38**: 182), and Wisconsin (Joyce, 1952, Ph.D. Thesis, Iowa State Coll., pp. 102–103).

This investigation was supported in part by a Science Faculty Fellowship from the National Science Foundation.—ALBERT G. CANARIS, *Zoology Department, Oregon State College, Corvallis, Oregon.*

PLATE I

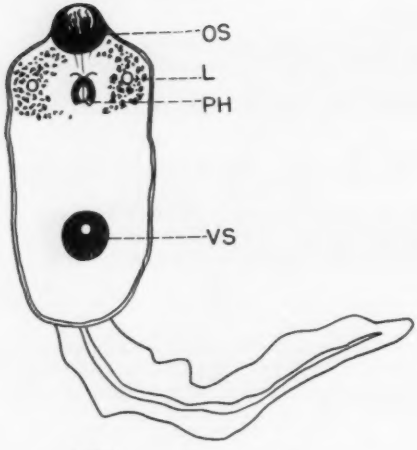
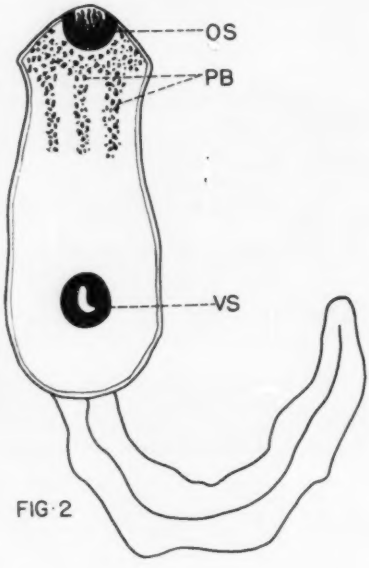


FIG. 1



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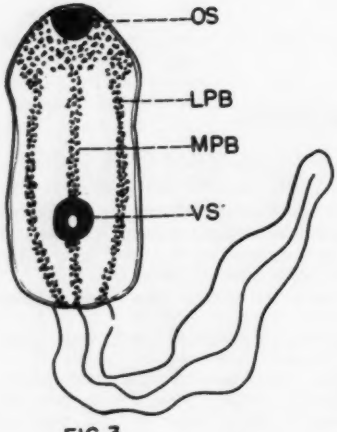

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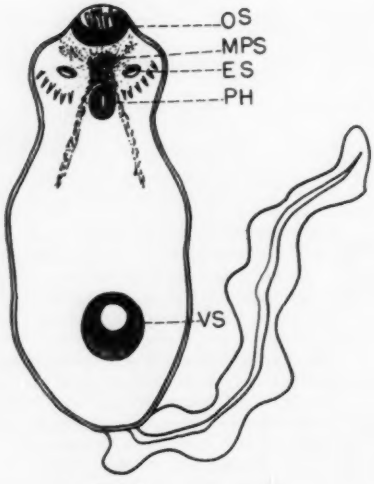
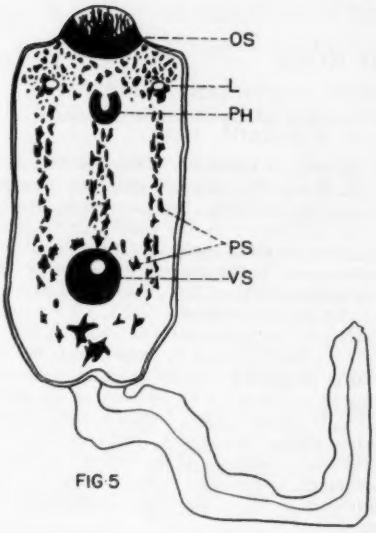
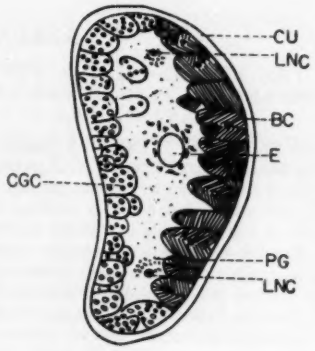


FIG. 4

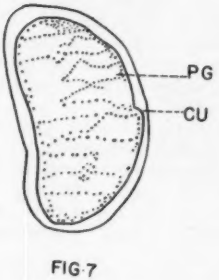
PLATE II



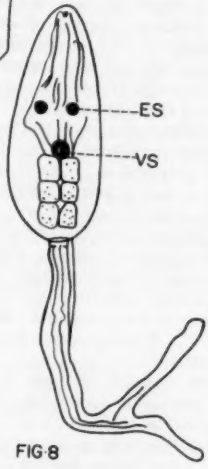
FIG·5



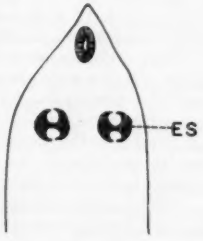
FIG·6



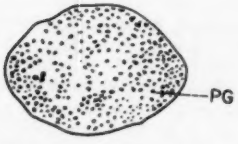
FIG·7



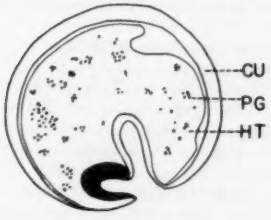
FIG·8



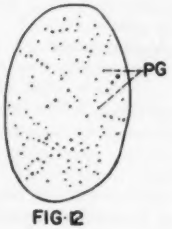
FIG·9



FIG·10



FIG·11



FIG·12

RESEARCH NOTE

THE AXENIC CULTIVATION OF TWO REPTILIAN PARASITES, *ENTAMOEBA TERRAPINAE* SANDERS AND CLEVELAND, 1930, AND *ENTAMOEBA INVADENS* RODHAIN, 1934.

While Stoll (1957, J. Parasit. **4** (supp.): 6) was first to report the axenic cultivation (as defined by Dougherty, 1959, Ann. N. Y. Acad. Sci. **77**: 27–54) of *Entamoeba invadens*, the axenic cultivation of *Entamoeba terrapinae* has not, to the writer's knowledge, been previously recorded. Recently it has been found that a medium similar to one devised by the writer for the axenic cultivation of trichomonads is suitable for culturing both of these amoebae (Diamond, 1957, J. Parasit. **43**: 488–490). The advantages of this medium over Stoll's are that it is clear and free of gross particulate matter, is easily prepared, and supports growth of large numbers of parasites.

The medium is composed of trypticase (BBL), 2.0 g; yeast extract (BBL), 1.0 g; maltose 0.5 g; L cysteine hydrochloride, 0.1 g; L ascorbic acid, 0.02 g; potassium phosphate, monobasic, 0.08 g; potassium phosphate, dibasic, anhydrous, 0.08 g; distilled water to make 90 ml; horse or sheep serum (inactivated 56 C, 30 min.), 10 ml. It is prepared in the following stepwise manner: Dry ingredients dissolved in water; pH adjusted to 7.0 to 7.2 by addition of 1N sodium hydroxide; mixture autoclaved 10 min, 15 lbs, 121 C; when cooled, serum added; medium transferred aseptically in 10-ml aliquots to screw-capped tubes; stored at 4 C until used.

The *E. terrapinae* used, strain 165, was isolated from a terrapin (*Pseudomys?*) in 1947 by A. M. Griffin and W. G. McCarten, George Washington University. The *E. invadens*, strain PZ, was isolated from a snake in 1945 at the laboratory of H. L. Ratcliffe, Philadelphia Zoological Garden; the identity of the host is unknown.

Both amoebae were originally isolated in xenic culture. Source materials for the present axenic cultures were obtained from cultures which had been monoxenized in our laboratory with the Culbertson strain of *Trypanosoma cruzi* (technique of Phillips, 1950, Sci. **111**: 8–9).

Attempts to establish axenic cultures by initially introducing amoebae and their trypanosome associates into the medium prepared as above resulted in the cultures dying out between the 3d and 5th subculture. Success was achieved only after the amoebae were first passaged serially through medium to which a small amount of agar had been added.

The procedure finally adopted for establishing axenic cultures is as follows: One ml (approximately 50,000 amoebae) of a 7-day-old amoeba-trypanosome culture is introduced into the bottom of a tube of medium containing 0.05 percent agar (Difco). Subcultures are made every 2 weeks for the first 12 weeks and weekly thereafter; this is accomplished in each instance by transferring 1 ml of the culture to a tube of fresh medium. Incubation is carried out at room temperatures which do not go below 25 C. Trypanosomes originally introduced with the amoebae disappear between the 2d and 3d transfer.

Beginning with the 7th transfer, attempts are made to establish the cultures in agar-free medium; usually the 1st attempt is successful. The routine for handling the amoebae in this phase of culturing is the same as outlined above.

It is of interest to note here that a culture of the PZ strain of *E. invadens* obtained from Dr. Stoll's laboratory in a bacteria-free but not axenic state (fresh liver cells were incorporated in the medium) could likewise be axenized in our medium only after passage through medium to which agar was added.

Axenic clones of *E. invadens* have been established. This has been achieved by microisolating single trophozoites in microcultures (technique of Rees, 1942, Am. J. Trop. Med. **22**: 487–492). After a week of incubation the resulting progeny of each isolate is transferred to a macroculture. The medium with agar is used for the microcultures and the initial series of macrocultures. No attempt as yet has been made to obtain clones of *E. terrapinae*.

Details concerning development of the amoebae in culture are to be published later. (The technical assistance of Ida Louise Bartgis is gratefully acknowledged. Preliminary studies on *E. invadens* were carried out while the writer was at the Animal Parasite Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland).—LOUIS S. DIAMOND, *Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.*

RAILLIETINA DATTAI N. SP. FROM POULTRY (*GALLUS GALLUS DOMESTICUS*) IN INDIA

P. K. SINHA
Assistant Professor of Parasitology, Bengal Veterinary College, Calcutta, India

Some specimens of cestodes from a hen kept at the Government Poultry Farm, Patna, were obtained for identification. A study of 8 specimens, of which 6 were complete, showed that they represented a new species.

Family Davaineidae Fuhrmann, 1907
Subfamily Davaineinae Braun, 1900
Genus *Raillietina* Fuhrmann, 1920
Subgenus *Raillietina* Stiles and Orleman, 1926
(= Ransomia Fuhrmann, 1920)

Raillietina (*Raillietina*) *dattai* n. sp.

Morphology: Length varies from 15 to 20 cm; maximum breadth 2 to 3 mm. Scolex measures 370 microns long and 400 microns wide. Suckers armed and each one bears 10 rows of hooks very small in size. Rostellum measures 70 microns in length and armed at its base with a double row of innumerable hooks 8 to 10 microns in length, hooks of anterior row slightly larger than posterior hooks. Fully mature segments 0.28 mm long and 1.8 to 2 mm broad, gravid segments 0.9 mm long and 2.8 mm broad. A single set of generative organ in each segment. Genital pores unilateral and open slightly anterior to middle of lateral margin of segments. In gravid segment opening appears to be right at middle. Testes 23 to 25 in each segment, 6 to 8 poral and 16 to 19 aporal. Cirrus sac very prominent and resembles shape of an hour-glass, very muscular and measures 250 microns in length and 70 microns in breadth; reaches excretory vessel. Cirrus unarmed. Vas deferens lies coiled along anterior margin of segment in a straight line and reaches near middle of segment. Seminal vesicle was not traceable. Ovary fan-shaped and much lobed measuring 210 microns × 270 microns, placed at middle of segment slightly anteriorly; vitelline gland compact and occupy posterior aporal corner of ovary. Vagina a muscular tube. Receptaculum seminis was not discernible. Uterus gives rise to 80 to 100 egg capsules which extend laterally beyond excretory vessels. Each capsule contains 6 to 12 eggs; egg measures 29 to 32 microns.

Host: Gallus gallus domesticus.
Location: Small intestine.
Locality: Patna, India.
Type specimens are preserved in author's collection.

DISCUSSION

The new species under consideration has been compared with 82 known species of the genus *Raillietina* subgenus *Raillietina,* and it has been found to have the greatest affinity to *Raillietina* (*Raillietina*) *volzi* Fuhrmann, 1905, of fowl. However, there are certain points by which *R.* (*R.*) *dattai* can be readily distinguished from *R.* (*R.*) *volzi.* The description and measurements of *R.* (*R.*) *volzi* given by Fuhrmann (1905) and Johri (1934) show that there is very little variation in cases of the number of testes, 30 to 32; the shape of the cirrus sac, pyriform; and the size of the egg, 20 to 25 microns. On these points *R.* (*R.*) *dattai* differ from *R.* (*R.*) *volzi* by having 23 to 25 testes, hourglass-shaped cirrus sac, and eggs 29–32 microns in size. The shape of the cirrus sac is a character of much systematic importance. Collection and permanent preparation of the specimens were done by standard techniques, and in all the specimens the shape of the cirrus sac is so constant that it appears to be a very stable character. Regarding other fea-

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tures, *R.* (*R.*) *dattai* lies within the wide range of variation observed in case of *R.* (*R.*) *volzi,* and hence, those features are of little significance for the purpose of identification.

The species has been named after Dr. S. Datta, formerly Director, Inidan Veterinary Research Institute, Izatnagar (U.P.), India.

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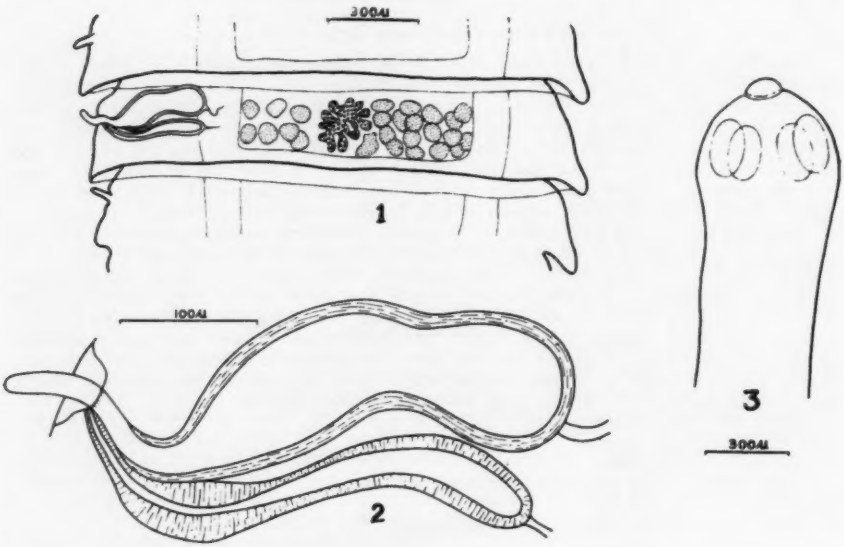

EXPLANATION OF PLATE

Raillietina (*R.*) *dattai* n. sp.

FIGURE 1. Mature proglottid showing ovary, testes, cirrus sac, and vagina.
FIGURE 2. Enlarged view of cirrus sac with protruded cirrus and vagina.
FIGURE 3. Scolex showing rostellum, suckers, and rostellar hooks.

A NEW DILEPIDID CESTODE, *MASHONALEPIS DAFYDDI* N. G., N. SP., FROM THE GRAY HERON, *ARDEA CINEREA* L.

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One complete cestode and several incomplete specimens were recovered from the intestine of a gray heron shot at the Henderson Fisheries Research Station, Mazoe, Southern Rhodesia, in March, 1959.

The worm was fixed in hot Bouin-Hollande and stained with Kirkpatrick's carmalum. Transverse and frontal sections were cut of mature proglottids using the polyethylene glycol distearate embedding technique (Steedman, 1957). This cestode represents a new genus for which the name *Mashonalepis* is proposed. All measurements are in millimeters unless otherwise stated.

Mashonalepis n.g.

Diagnosis: Dilepidinae Fuhrmann, 1907; scolex with unarmed, weakly muscular suckers; rostellar hooks in single row. Genitalia single in each proglottid; genital pores unilateral; genital atrium partly surrounded by sphincter. Genital ducts dorsal to poral excretory canal. Testes numerous, posterior, lateral and anterior to ovary. Cirrus sac weak; cirrus unarmed; internal and external seminal vesicles absent. Gravid uterus deeply lobed. Excretory system with one pair of longitudinal vessels.

Mashonalepis dafyddi n.sp.

Specific description: With characters of the genus. Body elongate; 252 long. Proglottids craspedote. Immature proglottids 0.12 long by 0.38; mature 0.37 by 1.67; gravid 0.84 by 1.67. Scolex small; 0.33 diameter with four shallow, unarmed, circular suckers 0.12 in diameter (fig. 1). Rostellum 0.14 long by 0.10; rostellar sac 0.18 by 0.14 maximum diameter; 16 rostellar hooks, 46 to 48 microns long, arranged in single row (fig. 2). Neck 0.62 long by 0.19. Strobila weakly muscular; inner longitudinal muscles poorly developed; a few transverse fibres occur in inter-proglottidal region. Excretory system with single pair of longitudinal vessels; maximum diameter 0.08; transverse connecting vessel present in posterior region of each proglottid. Genital pores unilateral, marginal, situated in anterior half of proglottid and 40 to 60 microns in diameter. Assuming that uterus arises ventral to ovary, genital pores lie on right side of strobila; terminal genital ducts pass dorsal to excretory vessel. Anlagen of male genitalia evident 21 behind scolex; that of testes 42. Number of testes variable (42 to 65); irregularly oval in shape; up to 62 microns diameter. Testes posterior, lateral and, in aporal region of proglottid, anterior to ovary. Vas deferens a coiled mass in front of poral lobe of ovary; continuing as a sinuous duct, anterior to vagina. External and internal seminal vesicles absent. Cirrus sac inconspicuous, weakly muscular, 0.16 to 0.19 long by 0.07 to 0.08 Ejaculatory duct 21 to 25 microns in diameter, opening via small unarmed cirrus. Cirrus up to 60 microns long by 48 to 53 microns wide. Proximal part of genital atrium surrounded by sphincter, 0.10 to 0.11 long by 0.11 to 0.12 (figs. 3 and 4). Atrium narrows to non-muscular, eversible region opening at gentital pore. Ovary bilobed, follicular, median; 0.82 maximum diameter. Vitellarium irregular, 0.08 to 0.10 by 0.10 to 0.11 diameter; median, posterior to receptaculum and Mehlis' gland. Mehlis' gland conspicuous, 0.12 to 0.15 long by 0.10 to 0.14, situated to right or left or on either side of receptaculum. Vagina opening into muscular bulb at atrium, posterior to cirrus. Initially 11 to 14 microns diameter, vagina expanding to 50 to 54 microns before narrowing to 32 microns at level of excretory vessel. Receptaculum seminis pyriform, 0.09 to 0.12 long by 0.07 to 0.08; median, dorsal to ovary (fig. 5). Uterus arising as short-stemmed T, ventral to anterior follicles of ovary; irregular lobes arising from horizontal limbs filling anterior and posterior regions of proglottid (figs. 6 and 7). Uterus dorsal to poral excretory vessel;

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ventral to aporal vessel. Eggs 49 to 53 microns diameter; embryos 25 microns; embryonic hooks 16 to 18 microns long.

Host: *Ardea cinerea* L.

Location: Anterior part of intestine.

Locality: Henderson Fisheries Research Station, Mazoe, Southern Rhodesia.

Type specimen: To be deposited in the collection of the British Museum (Natural History).

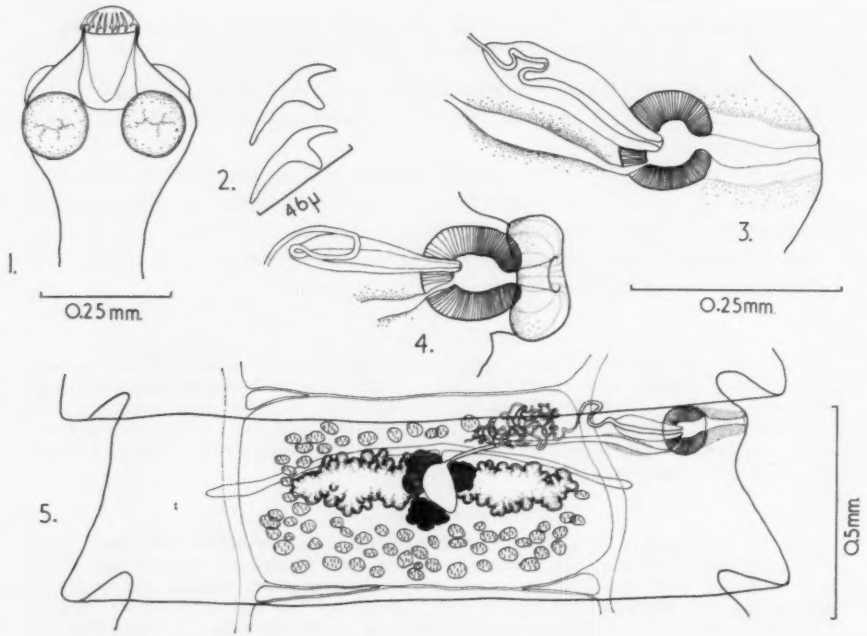

FIGURE 1. Scolex of *Mashonalepis dafyddi*, n.g., n. sp.
FIGURE 2. Rostellar hooks.
FIGURE 3. Genital atrium showing musculature and the openings of the cirrus and vagina.
FIGURE 4. Genital atrium partly everted.
FIGURE 5. Mature proglottid.

DISCUSSION

Of some 48 genera that have been assigned to the sub-family Dilepidinae Fuhrmann, 1907, the following 8 have been described with a distinctive musculature associated with the genital atrium or the vagina: *Pentorchis* Meggitt, 1927; *Valipora* Linton, 1927; *Ophiovalipora* Hsü, 1935; *Pseudangularia* Burt, 1938; *Bakererpes* Rausch, 1947; *Diagonaliporus* Krotov, 1951; *Neoangularia* Singh, 1952; and *Neoliga* Singh, 1952.

Three of these genera, *Pseudangularia, Neoangularia,* and *Neoliga* are typified by a dumbbell shaped structure surrounding the junction of the vagina and the receptaculum seminis which Burt (1938) considered to be a double vaginal sphincter. All 3 genera have alternating genital pores. According to the description and figures given by Coil (1950) for *Ophiovalipora minuta,* the genus has a vaginal sphincter and the rostellar hooks are arranged in a double row. Fuhrmann (1932)

stated that *Pentorchis* has a sphincter muscle surrounding the genital atrium. The number and distribution of the testes are, however, quite different from the present material. *Diagonaliporus* was erected by Krotov (1951) for forms with a sphincter surrounding the genital atrium, the position of which gradually changes from side to side over the ventral surface. According to Wardle and McLeod (1952), *Bakererpes* has a large muscular area surrounding the genital atrium, but the atria bear spines and are regularly alternating.

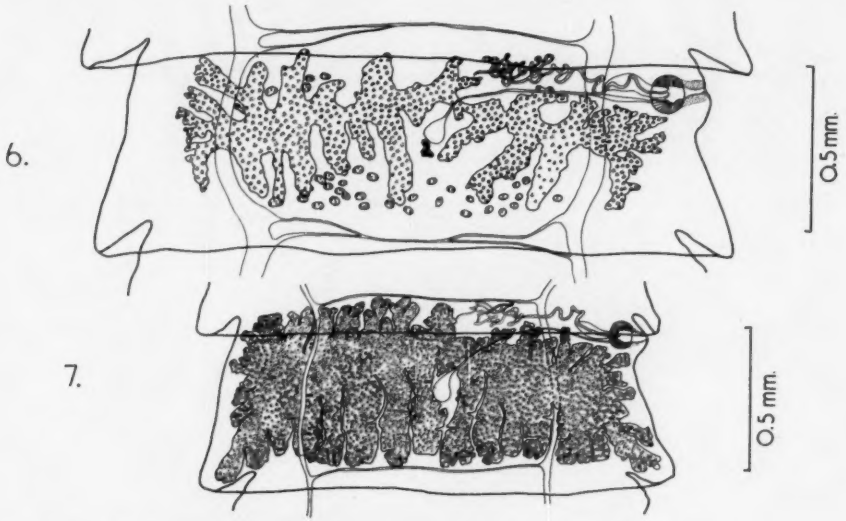

FIGURE 6. Semi-gravid proglottid showing expanding uterus of *M. dafyddi*.
FIGURE 7. Gravid proglottid.

Mashonalepis shows an affinity to the genus *Valipora*, since both have muscular genital atria, unilateral genital pores, and a single row of rostellar hooks. Linton (1927) gave inadequate descriptions of *V. mutabilis*, the type species, and *V. parvispine*. Although abundant material was available, Linton was unable to give definite data on the number and size of the rostellar hooks or the number of testes. From the figures given by Linton it would appear that in both *V. mutabilis* and *V. parvispine* the whole genital atrium is surrounded by muscular walls. In *Mashonalepis* the musculature is confined to an almost spherical, proximal sphincter. The distribution of the testes in the 2 genera is also different. In the present material some of the testes are pre-ovarian, while in *Valipora* the testes are dorsal to the ovary (*mutabilis*) or posterior (*parvispine*). *Mashonalepis* has (from an examination of sectioned, mature proglottids) but 1 pair of longitudinal excretory vessels ventral to the genital ducts. *Valipora* has both dorsal and ventral vessels between which the genital ducts pass.

The present material is unlike any of the above genera; therefore, a new genus *Mashonalepis* is erected with *M. dafyddi* as the type species.

SUMMARY

A new cestode, *Mashonalepis dafyddi* n.g., n.sp., from the gray heron, *Ardea cinerea* L., is described and compared with other genera of the sub-family Dilepidinae Fuhrmann, 1907, which have a distinctive musculature associated with the genital atrium or vagina.

ACKNOWLEDGMENT

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RESEARCH NOTE

STIMULATION OF CYSTICERCUS EVAGINATION BY MEANS OF SURFACTANTS.

In the light of several papers published recently on the activation and emergence of tapeworm larvae (Sawada, T., 1959, Exp. Parasit. **8:** 325–335; Rothman, A. H., *Ibid.* 336–364) the authors wish to record an observation made some years ago. Since the activity of bile salts in stimulating the evagination of cysticerci is well known (Edgar, S. A., 1941, Tr. Am. Micr. Soc. **60:** 121–128), an attempt was made to produce this effect using other surface-active agents. Cysticerci, of undetermined species, were removed mechanically from delicate cysts in the liver of white-tailed deer, *Odocoileus virginianus* Boddaert. In preliminary trials it was found that these larvae readily evaginated when immersed in solutions of several commercial household detergents, including "Fab" and "Joy." Solutions prepared from a bar of household soap exhibited a definite, but lesser, activating effect. No attempts were made to elaborate these findings on a quantitative basis, or to determine whether this means of inducing evagination lessened the viability of the larvae. Smith (1955, J. Parasit. **40** (supp.): 41–42) found that cysticercoids of *Raillietina cesticillus* frequently evaginated in detergent solutions. The use of artificial detergents in stimulating the evagination of taeniid larvae, does not seem to have been reported hitherto.—W. C. CAMPBELL, *University of Wisconsin, Madison, Wis.* (present address: *Merck Institute for Therapeutic Research, Rahway, N. J.*) AND T. RICHARDSON, *University of Wisconsin* (present address: *Bureau of Commercial Fisheries, University of California, Davis, Calif.*).

CYSTICERCOSIS AND HYDATID DISEASE OF CATTLE IN KENYA

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Many surveys have been conducted in different countries on the incidence of *Cysticercus bovis* and *Echinococcus granulosus* cysts in bovine carcasses. Abattoir findings of these two infections were given in the Annual Reports of the Department of Veterinary Services, Kenya (1954–58); the figures ranged between 20 and 35 percent for *C. bovis;* higher figures were for stock from African areas. The incidence of *E. granulosus* was 17 to 47 percent.

Articles from other countries usually refer to only one condition. A comprehensive survey of the literature on occurrence and other aspects of cysticercosis was made by Viljoen (1937), who gave infection rates from most countries in the world. Since then, figures published include those of Jepson and Roth (1952) who reported less than 1 percent in Denmark in 1948, Silverman (1955) who found varying rates of 0.81 to 3.47 percent at different abattoirs in the United Kingdom, Simitch and Nevenitch (1955) who recorded 10 to 20 percent infection with *C. bovis* in different parts of Yugoslavia, and Peel (1953) who found 38.4 percent of 1,000 N'dama cattle infected at abattoir inspection in Sierra Leone.

Pullar and Marshall (1958) who surveyed a large number of cattle in Australia for hydatid disease, recorded an overall 12 percent infection. Cyprus had the highest recorded incidence with up to 100 percent affected (Cyprus Dept. of Agr., 1957), while up to 90 percent of Yugoslavian stock was infected in some parts of the country (Nevenić, 1953). The infection rate in different districts of Chile varied from 0.6 to 86.3 percent (Neghme et al, 1951), and in Italy there was a variation from 1.5 to 42 percent according to Altara (1953). Pipkin and his co-workers (1951) reviewed the incidence in man and animals in the Near East and quoted several previous papers which showed that there was a cattle infection rate of 8.4 to 47 percent in that area. On the other hand, the United States of America appeared remarkably free, since an annual average of only 2,837 livers were condemned for hydatidosis in the period 1950 to 1957 from an average kill of 20 to 26 million cattle (Poole, 1957). The total infection rate was probably higher, as Schwartz (1957) pointed out in a discussion of Poole's paper, the liver: lung ratio being at least 2:3, but this still gives a very low percentage compared with other countries.

The present study in Kenya on 1,000 head of cattle was made chiefly to discover whether cysts of *C. bovis* and *E. granulosus* could occur in the same beast, or whether some interference occurred due to their relatively close generic relationship. Taliaferro (1930) quoted Meyer and others who stated that complement fixation cross reactions could be obtained with sera from persons infected with *T. saginata* and hydatid cysts against the heterologous antigens. Other workers did not confirm this. Miller (1932) reported that *T. pisiformis* material given intraperitoneally to rats conferred a high degree of protection against *T. taeniformis.* Coudert and Coly (1956) found that the serum of *Taenia* carriers agglutinated

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particles sensitized with hydatid or *Taenia* antigen, although that of hydatid patients did not agglutinate particles sensitized with *Taenia* antigen. Kagan (1957) showed that many helminths had antigens which cross-reacted with heterologous sera. There was the possibility that some immunological cross reaction occurred in cattle due to a common antigen interfering with the establishment of either or both *C. bovis* and *E. granulosus*.

MATERIALS AND METHODS

The Kenya Meat Commission abattoir at Athi River was visited on 19 occasions between August 20 and December 3, 1958, and records were taken of cattle from 118 farms in the European farming areas of Kenya, 2 African District Council Ranches, and 2 African Reserves in North Kenya.

Each time animals were chosen at random, taking every 2d or 3d carcass reaching the slaughter floor, according to the speed of throughput, which depended on the size of beast being handled. The incidence of *C. bovis* was taken from the findings of routine meat inspection laid down in the Laws of Kenya (1955). These specify inspection of the ventral aspect of the tongue, extensive incisions into the external and internal muscles of mastication parallel to the lower jaw, inspection of the heart and oesophagus, 3 incisions into the muscles of the shoulder proximal to the elbow joint with several subcuts into each, and inspection of other sites exposed during the cutting and dressing, such as the brisket and hump (where present). Cysts found in lungs and livers were also recorded. A note was kept of whether cysts were macroscopically fresh or calcified.

E. granulosus were searched for in lungs, liver, heart and spleen. (The animals under survey were chiefly infected in lungs and liver; only two had hydatid cysts in the spleen and one a small calcified hydatid cyst in the heart muscle.)

RESULTS

Of the 1,000 animals examined, 317 had *C. bovis* and 255 *E. granulosus* cysts. The analysis of various aspects of the 2 infections is summarized in tables I and II.

TABLE I. *Incidence of* Cysticercus bovis *and* Echinococcus granulosus *cysts in 1,000 slaughter cattle.*

	CE	C	E	O	Total	Chi-Square	P
1. All cattle	96	221	159	524	1,000	5.5912	< 0.02
2. Farms with both	96	176	129	342	743	4.7367	< 0.05
3a. European only	71	198	145	490	904	1.316	> 0.2
3b. African only	25	23	14	34	96	4.319	< 0.05
4a. Males	38	160	105	351	684	3.3666	> 0.05
4b. Females	28	61	54	173	316	1.5795	> 0.2
5a. Exotic	29	89	60	239	417	0.7738	> 0.5
5b. Zebu	67	132	99	285	583	3.6256	> 0.05

CE, *C. bovis* and *E. granulosus* cysts present; C, *C. bovis* present, *E. granulosus* not found; E, *C. bovis* not found, *E. granulosus* present; O, no cysts of either parasite found.

Line 1 of table I shows the incidence of the 2 infections calculated for all cattle surveyed: 96 head had both parasites, 221 *C. bovis* only, 159 *E. granulosus* only, and 524 were free of cysts.

On the 122 separate areas from which stock were included in the survey, 72 had both *C. bovis* and *E. granulosus* infections, 20 had *C. bovis* only, and 18 *E. granulosus* only, while 12 were apparently clear of both infections. Due to the sampling technique it is likely that infection was not established for some farms because so few animals were examined. The incidence of infection on those with both conditions is given in table I, line 2. The figures here were 96 with both, 176 with *C. bovis* only, 129 with *E. granulosus,* and 342 with neither. A division of stock according to origin from European farms and African areas is given in line 3.

It was found that 68 males were infected with both types of cysts, 140 with *C.*

TABLE II. *Number of* C. bovis *cysts and incidence of living and calcified cysts, according to age of beast, and age incidence of* E. granulosus.

Age (years)	Total animals seen	No. of cysts				Av.	No. of animals with			*C. bovis* pct. positive	*E. granulosus*	
		1	2–6	7–20	21 and over		Living cysts	Calcified cysts	Both types		No. positive	Pct. positive
0 to 1	31	0	2	0	0	3.0	1	0	1	6.45	6	19.35
1 to 2	6	0	1	0	0	4.0	1	0	0	16.67	1	16.67
2 to 3	18	1	4	2	0	4.0	4	2	1	38.89	3	16.67
3 to 4	72	14	15	0	0	1.93	20	7	2	40.27	13	18.06
4 to 5	662	74	100	27	18	4.78*	161	32	26	33.08	178	26.89
5 to 6	56	7	8	4	0	3.79	15	1	3	33.93	10	17.86
6 to 7	42	5	5	2	0	3.0	6	3	3	28.67	14	33.33
7 to 8	45	2	6	1	0	3.55	6	3	0	20.00	7	17.50
8 to 9	33	3	7	1	0	3.45	7	3	1	33.33	11	33.33
10 and over	35	2	6	0	0	2.00	6	2	0	22.86	12	34.29
Totals	1,000	108	154	37	18	4.21	227	53	37	31.7	255	25.5

* Multiple infections with over 20 cysts per carcass were calculated as only 21, although number much higher but uncounted.

bovis only, 105 with *E. granulosus* only, and 351 were uninfected; the comparative figures for females were 28, 61, 54, and 173, respectively.

Finally the type of stock surveyed was analyzed and it was found that 29 head of exotic stock had both types of cysts, 89 had only *C. bovis*, 60 only *E. granulosus* and 239 neither; the zebu analysis was 67, 132, 99, and 285, respectively.

In 3 analyses, the presence or absence of one type of infection influenced that of the other at the 5 percent level. These were for total stock, stock from farms with both diseases, and cattle from African areas alone.

Table II gives the age incidence of *C. bovis* and *E. granulosus* cysts. Age was estimated on dentition. It is well known that the teeth of zebus erupt later than those of exotic stock, and 6 months allowance was made for this. Aging of cattle 6 to 9 years old was made according to the degree of wear on the incisors, and all animals showing severe wear, with only stumps of teeth left, were judged to be 10 years or older.

No significance was found in the fall in incidence of *C. bovis* or the rise of *E. granulosus* with increasing age.

Cysts at various ages are also shown in table II. Of the total 317 head infected with cysticercocis, 227 (71.6 percent) had only fresh cysts, 53 (16.7 percent) only calcified cysts, and 37 (11.7 percent) both types. It was not practicable to test the uncalcified cysts for viability.

In 108 (34.1 percent) of the animals with *C. bovis*, only 1 cyst was found on routine inspection; 154 (48.6 percent) had 2 to 6 cysts; 37 (11.7 percent) had 7 to 20 cysts, and 18 (5.6 percent) were condemned as cases of generalized cysticercosis. Except that carcasses with only 1 cyst are shown separately, the above grouping of cyst frequencies is in accordance with the various treatments of infected meat required under the Meat Inspection Rules (Kenya, 1955). The number of cysts in relation to the animals' ages is shown in table II which also shows the average number of cysts per carcass; cases of multiple infection have been calculated as containing only 21 cysts. The average was much higher but no precise count was made on the 18 beasts involved.

Li and Hsü (1951) investigated the frequency distribution of certain parasitic helminths in their respective hosts. A similar distribution for *C. bovis* was found in this series.

Pullar and Marshall (1958) and others have recorded the organ incidence of

hydatid disease. My figures for the organs of 255 infected cattle were as follows: liver, 122; lungs, 87; both liver and lungs, 43; liver, lungs, and spleen, 2; heart, 1.

The total liver to total lung ratio was 1 : 0.79.

The total liver to total infected ratio was 1 : 1.52.

The liver only to lung only ratio was 1 : 0.71.

DISCUSSION

Ginsberg et al (1956) have discussed the problem of the inspection incisions required to ascertain with reasonable certainty the presence of *C. bovis*. It is likely that a number of cysts were not seen by routine inspection; but since the survey had to be as wide as possible for the investigation of the main object, such limitations were essential to avoid unnecessary damage to carcasses. Some animals are therefore likely to have been positive but recorded as negative in this survey, and others to have been more heavily infested than recorded.

Analysis of the concurrent infection of measles and hydatid showed in 3 cases that there was some interaction between them at the 5 percent level of significance. On the size of sample in this survey, a significance of 1 percent would have had to be found before mutual interference could have been inferred.

It would appear from an examination of table I, line 3b, that there is a possibility that if an animal becomes infected with *C. bovis* it is more likely to become infected with hydatid cysts, or vice versa. The figures for the African areas are relatively small, and the incidence of the 2 conditions is significantly higher than in the European areas.

Koch and Steinitz (1951) calculated the expected incidence of simultaneous infections in humans with *Ascaris lumbricoides* and *Trichuris trichiura* and found a significant difference between expected and actual incidence. Using a similar method of calculation the expected and actual numbers for total stock, 81 and 96; farms infected with both, 62 and 96; European owned stock, 72 and 71; African owned stock, 17 and 25; males, 27 and 68; females, 23 and 28; exotic type cattle, 44 and 29; and zebus, 19 and 67. Thus, excluding the figures for European and exotic stock, the actual incidence is higher than expected in every case, which tends to support the argument that the 2 parasites do not interfere immunologically but may in fact have the opposite effect.

Ginsberg (1956) and others have found that sex appears to have some influence on the incidence of *C. bovis*. Campbell (1939) stated that sex differences were important in the susceptibility of rats to *Cysticercus crassicollis* where only 60 to 80 females became infected for every 100 males. Although the present work showed an infection rate in males of 33.3 percent and in females of 28.16 percent, this difference is not statistically significant.

Results in table II pertaining to the effect of age on the presence of *C. bovis* are not as clear cut as Peel (1953) suggested. The decrease in incidence with age was not statistically significant. Figures are also shown of living and calcified cysts. While it was not practicable to test for viability, all cysts which were not calcified could not have been dead for very long. On this basis fresh infections were found in relatively old animals. Penfold and Penfold (1937) found a solid immunity to reinfection could be created against *C. bovis*, or might occur naturally if animals are subjected to a heavy initial infection. The fact that Urquhart (1959) has been

unable so far artificially to infect cattle over 9 months old in Kenya leads one to speculate that stock in Kenya is generally exposed to infection at a very early age and, as stated by Penfold and Penfold (1937), are resistant to reinfection. Cysts may, therefore, take very much longer to degenerate and calcify than was previously believed (Penfold, 1937). This point requires further investigation under conditions in Kenya. The author has also so far failed to infect adult stock with material infective to young calves; most of the animals so treated were found at slaughter to have been preinfected, but with only a very few cysts.

A high percentage of the hydatid cysts in the livers were calcified or in the early stages of degeneration as described by Pullar and Marshall (1958). A very much smaller proportion of lung cysts were undergoing degenerative changes.

The present survey revealed no significant sex difference of hydatidosis infection rate in contrast to Pullar and Marshall's findings in Australia where females showed more than double the incidence of males. The variation of age noted by the Australian authors was confirmed in the current survey in which the age incidences were as follows: under 4 years, 18 percent; 5 to 6 years, 26 percent; over 7 years, 28 percent. Again, the increase was not statistically significant. It is of interest to note that while the incidence of *C. bovis* decreases with age, that of the hydatid increases even if the above figures are insufficient to prove it conclusively.

SUMMARY AND CONCLUSIONS

One thousand head of cattle were examined for cysts of *C. bovis* and *E. granulosus*.

Tables are given on their incidence in European and African owned stock, according to sex, age, type, and other aspects of the two zoonoses.

The findings are discussed and the following conclusions drawn from the survey:

1. Cysticercosis and hydatidosis do not appear to cause mutual interference in cattle; i.e. the presence of one does not preclude that of the other, although the figures are strongly suggestive of some relationship between them.
2. Males were found to contain *C. bovis* cysts more often than females, but there was no difference between the sexes in the occurrence of hydatid cysts.
3. There was a slight decrease in incidence of cysticercosis with increased age, while the reverse was the case in *Echinococcus* infection, but neither figure was statistically significant in the current survey.
4. Even old cattle were found to harbor undegenerated cysticerci.

ACKNOWLEDGMENTS

Permission to publish this paper was given by the Director of Veterinary Services, Kenya. I wish to thank Mr. M. C. Round and Mrs. B. Rogoff who helped collect the data, the Kenya Meat Commission for facilities, Dr. Ginsberg and his staff for their co-operation, and the East African High Commission Statistical Department and Messrs. G. R. Scott and G. Knight for the statistical analyses.

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TRANSAMINASES OF TAPEWORMS*

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Although transaminases were identified in animal tissues as early as 1937, very few observations have been made on these systems in parasitic helminths. Daugherty (1952) found that homogenates of the liver fluke, *Fasciola hepatica,* contained very high transaminase activity. Using the formation of glutamic acid from alpha-ketoglutaric acid as an indication of transaminase, Daugherty demonstrated the utilization of 11 of 13 amino acids tested as amino donors. The quantity of glutamic acid formed was high when aspartic acid, isoleucine, leucine ,and valine were used; the activity was moderate with arginine, alanine, phenylalanine, tyrosine, methionine, and proline. Tryptophan gave questionable results, and lysine and cystine were inactive. In contrast to the high transaminase activity of liver fluke homogenates, Aldrich, Chandler, and Daugherty (1954) found the activity of homogenates of the tapeworm, *Hymenolepis diminuta,* to be relatively low, only a few amino donors being effective. In 3 systems, alpha-ketoglutaric acid-glutamic acid, pyruvic acid-alanine, and oxalacetic acid-aspartic acid, only 3 amino acids, aspartic acid, glutamic acid, and alanine were effective as amino donors.

MATERIALS AND METHODS

H. diminuta was obtained from Sprague-Dawley rats previously infected with 10 to 12 cysticercoids reared in the flour beetle, *Tribolium confusum. H. citelli* was reared in hamsters similarly infected with cysticercoids from flour beetles. These worms were used 5 to 7 weeks after establishing the worms in the vertebrate hosts. *H. nana* was reared in Princeton Swiss and C57 mice. Each mouse was infected by feeding a number of gravid proglottids with a medicine dropper. *H. nana* were recovered from the mice 13 to 16 days after infecting. In one experiment, *H. nana* reared in the squirrel, *Sciurus carolinensis,* were used. Host animals were killed by a blow on the head, the intestine removed, and the worms washed out by syringing with 0.9 percent sodium chloride solution. In the case of *H. nana,* the intestine was carefully opened for recovery of the worms. In each experiment, 4 to 7 worms of *H. diminuta* or *H. citelli* were used; with *H. nana,* 50 to 60 worms were used. The recovered worms were washed thoroughly in 3 changes of cold 0.9 percent sodium chloride, transferred with 1.0 ml of 0.05 M phosphate buffer (pH 7.6) to a chilled Potter-Elvehjem glass homogenizer, and homogenized in the cold. The preparation was brought to the required volume, usually 12 ml, with phosphate buffer, centrifuged at low speed for 5 minutes to remove large fragments, and the cell-free supernatant pipetted off for immediate use.

Transaminase activity was determined by the method of Giri et al (1952a, b), as modified by Zeledon (to be published elsewhere). The L forms of amino acids were prepared as 0.1 M solutions in 0.05 M phosphate buffer (pH 7.6). In a few instances when DL amino acids were used, the amino acids were made up in 0.2 M solution. Alpha-ketoglutaric acid was prepared as a 0.1 M solution with the pH adjusted to 7.6. Sodium pyruvate was made up to the same concentration having the same pH. In the case of the less soluble amino donors,

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stock solutions of lower concentration were prepared and larger aliquots were used in the assays; e.g., cytosine was 0.025 M, phenylalanine was 0.05 M, and adenine was 0.015 M. A saturated solution of tyrosine was used. The volume of the reaction mixture was kept constant by varying the amount of buffer solution added.

The reaction mixture contained 25 micromoles of amino donor (15 micromoles in the case of adenine), 25 micromoles of the keto acid, 0.5 ml of the tissue preparation (325 to 890 micrograms total nitrogen in the case of *H. diminuta*; 210 to 350 micrograms of nitrogen in the case of *H. citelli*), and phosphate buffer to a final volume of 2.0 ml. In assays with *H. nana*, 0.5 ml of the tissue preparation (containing 100 to 275 micrograms of nitrogen) was used, but the keto acid and amino acid additions were reduced one-half and the final volume of the reaction mixture was 1.0 ml. After 60 minutes incubation at 37°C, the reaction was stopped by the addition of an equal volume of absolute ethyl alcohol. After mixing, the tubes were centrifuged sharply and an aliquot of the supernatant fluid chromatographed on paper in one dimension with n-butanol, acetic acid, and water (4:1:5). The paper was dipped in 0.5 percent ninhydrin in 95 percent acetone, dried at 60°C, and the spots eluted in 5 ml of 75 percent ethyl alcohol containing 50 mg of copper sulfate per liter.

In each experiment, the following controls were used: (1) Reaction mixture with alcohol added before incubation; (2) reaction mixture without amino donor; (3) reaction mixture with a standard amount of glutamic acid or alanine added; (4) same as control (3) but with alcohol added before incubation. Recoveries were estimated from controls (3) and (4) which did not differ significantly. The average recoveries were 102 and 108 percent with glutamic acid and alanine, respectively. All assays were run in duplicate and the values obtained are reported as the mean of 2 experiments. Total nitrogen was determined by the method of Lang (1958).

RESULTS

The data on transaminations obtained with homogenates of the three species of tapeworms are presented in table I. It is obvious that these worms are very re-

TABLE I. *Transaminase activity of 3 species of* Hymenolepis. *Values are micromoles of amino acid formed/mgN/hour. The value "0" means that transaminase activity could not be detected.*

Amino donor	Alpha-ketoglut. → glutamic			Pyruvic → alanine		
	H. diminuta	*H. citelli*	*H. nana*	*H. diminuta*	*H. citelli*	*H. nana*
Alanine	5.0	4.5	0	—	—	—
Aspartic acid	17.7	28.6	31.9	3.1	0	0*
Asparagine	3.8	4.0	3.8	0.9	0	0
Glutamic acid	—	—	—	13.4	14.1**	8.5

The following compounds did *not* serve detectably as amino donors to pyruvate or alpha-ketoglutarate with any of these three tapeworm species: Glutamine, arginine, glycine, histidine, leucine, lysine, cystine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine, adenine, cytosine, and glucosamine.

* In one experiment with *H. nana* from squirrels, the value for aspartic acid was 6.3 and that for asparagine was 0.

** When the homogenate was frozen overnight the average value decreased to 8.1.

stricted in their capacity to carry out transaminations. Very few amino compounds serve as effective amino donors. It is of interest that these three closely related species of tapeworms can be distinguished by the presence or absence of transaminase activity when the total number of transaminations performed by homogenates of their tissues are so limited. *H. citelli* and *H. diminuta* can utilize alanine as an amino donor with alpha-ketoglutaric acid, whereas *H. nana* apparently cannot; *H. diminuta* can be distinguished from *H. citelli* by its ability to use aspartic acid and asparagine as donors of amino groups in the amination of pyruvic acid.

DISCUSSION

The present data on the transaminase activity of *H. diminuta* agrees essentially with the findings of Aldrich (1954) and Aldrich, et al (1954). These authors did

not test asparagine which has been found to serve as an amino donor to either of the keto acids tested. When compared to vertebrates (Awapara and Seale, 1952), insects (Kilby and Neville, 1957), *Ascaris* (Savel, 1955), and hemoflagellate protozoa (Zeledon, Ms. in preparation), the tapeworms examined in the present study have an extremely limited capacity for performing transaminations.

The finding that *H. nana* from squirrels can evidently carry out the amination of pyruvic acid with aspartic acid as the amino donor strongly suggests that the transaminases may be highly labile systems in their phenotypic expression. It would be of interest to determine whether this is an inducible system by experimental transplantation of worms from mice to squirrels and vice versa, a technique recently exploited in physiological studies on tapeworms (Goodchild, 1958; Schiller, 1959).

The very limited capacity of these worms to carry out transaminations supports the general concept that they may utilize synthesis to a relatively minor extent in satisfying amino acid requirements. Recent kinetic studies by others in this laboratory indicate that these and other tapeworms have a marked capacity for absorbing a variety of amino acids from the surrounding medium. If further study substantiates the idea that very little keto acid carbon of carbohydrate origin enters the amino acid pool, it may be suggested that carbohydrate metabolism of tapeworms closely approximates the "energy engine" type of metabolism so often described for organisms in general by elementary textbooks.

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THE ORAL TRANSPLANTATION OF INTESTINAL STAGES OF *TRICHINELLA SPIRALIS*

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Varying opinions have been expressed as to the infectivity of intestinal stages of *Trichinella spiralis*. Hemmert-Halswick and Bugge (1934) concluded from in vitro studies that trichinae which had completed moulting, at around 20 hours, were no longer capable of withstanding gastric juice, and, therefore, only those worms which had not completed moulting were infective for another host. In contrast to this, Gursch (1949) found active, sexually mature *Trichinella* in the stomach of rats on occasion. It was his opinion that these worms had either never reached the intestine or had gone from the intestine into the stomach.

Nolf (1937; 1938, unpublished, cited by Anderson, 1941) was able to recover muscle larvae from rats which had been inoculated either orally or by surgical means with gravid females. However, Anderson (1941) and Anderson and Leonard (1940) found that trichinae removed from the intestines of rats at intervals of 10, 15, or 20 hours after inoculation, and then introduced orally into normal rats, did not produce muscle infections. Furthermore, no muscle larvae were obtained from rats which received into the duodenum by surgery 24- or 30-hour females, but 36-, 42-, and 48-hour worms produced infections, the number of larvae recovered increasing with the length of the intestinal phase.

While investigating the transplantation of intestinal stages of *T. spiralis* in mice, this writer observed that some mice acquired muscle infections of larvae when inoculated per os with trichinae which had been in the small intestine of another host for more than 28 hours. In view of the above findings, it appeared that this phenomenon was worthy of further study. Therefore, an investigation was carried out to determine at what time in the intestinal phase of the life cycle *T. spiralis* could be successfully transferred per os from one host to another.

MATERIALS AND METHODS

The Swiss mice used in these experiments were from 18 to 32 g in weight, averaging 23 g. They were allowed Laboratory Chow before inoculation but were starved for up to 24 hours after inoculation. Water was always available ad libitum. All mice were from colonies infected with *Hymenolepis nana* and pinworms.

"Donor" mice were inoculated with muscle larvae obtained from digests of infected rats. These mice were not given feed for up to 24 hours prior to their being sacrificed in order to collect the intestinal worms. At various time intervals, donors were killed and the small intestine was removed. This was done from 1 to 4 hours before the actual transfer was made. The mucosa was scraped and the nematodes were isolated by standard procedures.

A known number of nematodes, in a minimum volume of saline, was introduced into the "receptor" mouse by means of a syringe with a blunted 18-gauge needle. The needle and syringe were flushed with saline to check for worms that remained. These were either inoculated again into the receptor or deducted from the original inoculum. The total volume, therefore, varied from 0.5 to 1.5 ml. The inocula contained approximately one-half to two-thirds females, the ratio of females to males apparently increasing with the age of the infection.

Examinations for muscle larvae were carried out 21 or more days after inoculation. If press

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preparations of diaphragm or other muscles proved negative, the mice were processed by the digestion procedure and the digested material was examined for larvae.

Representative receptor mice were killed on the day following the inoculation and were examined for the transplanted worms. Material obtained by scraping the small intestine was placed in a Baermann apparatus for isolation of the nematodes.

RESULTS

The recovery of muscle larvae was considered to be the criterion for a successful transplantation. The results of experiments to produce such infections are recorded in table I. The control mice received only muscle larvae which are designated as

Table I. The recovery of larvae from the musculature of mice inoculated per os with intestinal stages of Trichinella spiralis.

Inoculum		Recovery of larvae	
Interval* in hrs.	No. worms	No. mice	No. pos. for larvae
0	140-580	14	14
5 ± 1	200	3	3
24 ± 1	40-200	7	0
28 ± 1	30-85	5	0
48 ± 1	89-100	6	4
52 ± 2	30-200	8	7
72 ± 3	30-200	11	8

***The time interval from the initial inoculation of the muscle larvae into donor mice to the feeding of the receptor mice.**

"0" hours. Five-hour worms yielded infections which were essentially comparable to controls. No infections were obtained with worms of the 24- or 28-hour group. Intestinal trichinae, when transferred after 48, 52, or 72 hours produced larvae in the majority of the mice.

The smallest number of transplanted intestinal worms to yield a positive muscle infection was 30 trichinae of the 72-hour interval group. Therefore, mice which had received less than this number have not been included in the data.

The degree of development of *Trichinella* at different intervals has been described by other workers. Suffice it to say here, at 24 and 28 hours the majority of the worms were found to be within a cuticular sheath. Samples of 48-hour worms showed many females to be fertilized and with eggs in the early stages of embryonation. These

evidences of reproduction, of course, were more notable at longer time intervals.

In view of the above findings, it was desirable to demonstrate the transplanted worms in the intestines of the receptors. The recovery of post-copulatory females was considered to be indicative of eventual larvi-position and, therefore, a successful transfer. As can be noted in table II, no worms were obtained from mice of the

Table II. The recovery of transplanted Trichinella spiralis intestinal stages from the small intestines of receptor mice.

Inoculum		Recovered worms			Mice	
Interval* in hrs.	No. worms	Time in host, hrs.	Sex M	Sex F	Total no.	No. pos. for F.
24 ± 1	76-150	19	0	0	3	0
28 ± 1	45-85	15-17	1	0	2	0
48 ± 1	89-91	19	0	4	4	2
52 ± 2	35-90	15-23	0	4	2	1
72 ± 3	35-95	17-21	1	6	6	1

***The time interval from the initial inoculation of the muscle larvae into donor mice to the feeding of the receptors.**

24-hour group and only 1 male was obtained from the 28-hour group. Female worms were recovered from representatives of the subsequent groups. Only 1 of the 6 mice which made up the 72-hour interval was found to be positive for intestinal stages. In all cases, the worms which were recovered were mature and the females had been inseminated. The male worm of the 72-hour group was inactive, while all the other recovered nematodes were active.

DISCUSSION

The results of this investigation demonstrate that, under certain conditions, intestinal trichinae can be transferred orally from one mouse to another and, in the recipient, may give rise to muscle-invading larvae. Furthermore, these experiments extend the time-intervals for such transfers beyond that previously reported by Anderson (1941) and Anderson and Leonard (1940). Although Matoff (1956, 1957) has also carried out peroral transfaunation studies, his method of transferring intestinal trichinae was primarily by feeding the intestines of the donors (guinea pigs, mice, rats) to receptors (dogs, guinea pigs, rats, rabbits) while in the present work, transfers were always made with isolated worms and only mice were used as hosts.

The observation that 5-hour intestinal trichinae can produce good infections in animals is in agreement with the findings of Anderson (1941). It should be noted that Hemmert-Halswick and Bugge (1934) found such worms to be as resistant to digestion in vitro as muscle larvae.

In analyzing the data for the 24-hour and later groups, one is confronted with 2 major factors: (1) the establishment of the transferred worms in the receptor host, and (2) the production of muscle larvae.

The data presented above suggest that trichinae in mice 24 or 28 hours beyond the infective muscle stage are not capable of becoming established in other mice. Contrary to this, Matoff (1956) recovered 6 larvae from the diaphragm of a rat which was fed the small intestine of a mouse containing "1 tägig (22 St.)" intestinal trichinae. This adds to the significance of our observation and indicates that further experimentation on this matter is desirable and of importance.

With regard to the 24- and 28-hour intervals, it is important to note that Gursch (1949) found the majority of intestinal trichinae after 20 hours in rats in the lumen of the intestine; by 2 days, most of the worms were again embedded in the mucosa. The occurrence of the nematodes in the lumen may be part of the reproductive processes (Gursch, 1949). After 24 or 28 hours in the intestine of mice, the females are not as yet inseminated (Wu and Kingscote, 1957), and, as has been pointed out above, moulting has not been completed. Therefore, transplanted trichinae at this phase of the life cycle cannot produce muscle-invading larvae without first becoming established in the receptor host, completing development to maturity, and undergoing copulation. We are assuming here that *Trichinella* mates in the adult stage. Contrary to what others have believed, recent observations by Wu and Kingscote (1957) indicate that this nematode mates after the final moult.

In this study, it was possible to recover transplanted nematodes or muscle larvae from some of the mice of the 48-, 52-, and 72-hour groups. Although Matoff's (1956, 1957) technics and hosts differed from ours, it should be noted that he also did not recover transferred intestinal trichinae or muscle larvae from all receptors. Moreover, he (1956, p. 229) recognizes host differences in his transfaunations and states: "The muscle invasion obtained in this way proved to be weak when the infective material was taken from rats and mice, and stronger when it derived from guinea pigs."

The discrepancies between numbers of mice yielding muscle larvae and the numbers of mice positive for intestinal females raises the question as to the actual establishment of the transferred worms in the new host. This is particularly the case in the 72-hour group where females could be recovered from only 1 of the 6 mice which were examined, while 8 of 11 mice inoculated with worms of the same interval were positive for muscle larvae. This raises the question as to whether transplanted, inseminated females could release embryos without becoming established in the intestinal mucosa of the receptor, and then be subject to elimination from the host in a matter of hours.

It is known that larval trichinae may be deposited quite early in experimental animals. Heller (1933) found larviposition taking place in rats and cats as early as 80 to 90 hours after infection. More recently, Gould et al (1955) were able to recover larvae from the blood of rats and rabbits at 114 hours after inoculation of muscle trichinae. The data presented here are insufficient to permit more than speculation. However, from the foregoing, it seems possible that at least the most developed embryos in some of the females of the 72-hour group could have been deposited and invaded the muscles. As a matter of fact, Nolf's (1938, unpublished, cited by Anderson, 1941, p. 22) studies on intraperitoneal injections of living gravid *Trichinella* also indicate that only those larvae which were contained in the uterus of the worm at

the time at which they were introduced had ultimately reached the musculature of the host.

Our findings may be applied to a more pragmatic conclusion. Spindler (1953) demonstrated the transmission of *T. spiralis* to pigs through feces from donors 1 to 28 days after the donors (pigs, dogs, cats, albino rats) were fed infected pork. Olsen and Robinson (1958) and Zimmermann et al (1959) found that *Trichinella* could be transmitted through feces particularly when the feces were from the first 24-hour period after the donors had received infectious material. Therefore, what has been observed in the study reported here may be of importance in the epidemiology of trichinosis.

SUMMARY

1. A study has been carried out to determine at what time in the intestinal phase *Trichinella spiralis* can be transplanted from one mouse to another per os.

2. Muscle infections were obtained in all mice fed 5-hour worms. These infections were comparable to those in control animals.

3. Neither intestinal nor muscle infections were demonstrated for worms transplanted 24 or 28 hours beyond the infective muscle stage.

4. Muscle larvae were recovered from the majority of mice inoculated with 48-, 52-, or 72-hour intestinal trichinae. Although transplanted worms were recovered from the small intestines of some mice of these time intervals, a correlation could not be made between the intestinal and muscle infections.

5. It is suggested that some of the 72-hour intestinal females may have deposited larvae without becoming established in the intestinal mucosa of the receptor mice.

6. The results of the experiments reported may be of epidemiologic importance.

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SYPHACIA SRIVASTAVI N. SP. FROM DOMESTIC PIG IN INDIA

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While studying the incidence of helminths in pigs, a new species of Oxyurid was recorded from their stomach. Visceral organs were collected from the pork market and examined for helminths. Two stomachs were found infected with the nematode and in both cases only female worms were recovered. No male worm was detected. Altogether 6 specimens were collected intact besides a few damaged ones. The descriptions and measurements of the female are based on those 6 specimens.

Family Oxyuridae Cobbold, 1864
Sub-family Oxyurinae Railliet, 1916
Genus *Syphacia srivastavi* n. sp.

Male. Absent.

Female. (Plate—I) Length 5 to 5.7 mm, average length 5.3 mm. Breadth at anterior end 0.064 to 0.085 mm, average 0.083. Maximum breadth 0.29 to 0.34 mm, average 0.3 mm. Body almost straight, posterior end slightly curved; cuticle transveresely striated with striations 10 microns apart from one another near anterior end. Such striations absent in tail which is very elongated and pointed at tip. Cuticle at anterior end forms a cephalic vesicle. Mouth surrounded by 3 lips each with 2 lobes; apparently no buccal capsule; mouth leads to a short tubular pharynx continuous with oesophagus. Muscular oesophagus club-shaped anteriorly with large spherical bulb posteriorly with vulvular apparatus, separated by a constriction. Maximum width of prebulbar oesophagus 0.057 mm, width of oesophageal bulb 0.114 mm. Nerve ring surrounds oesophagus 0.328 mm from anterior end. Whole length of oesophagus including pharyngeal portion and posterior bulb varies from 0.57 to 0.71 mm, average 0.65 mm, one-ninth to one-eighth of body length. Intestinal tube straight, narrows down near slightly dilated rectum and opens through anus 0.71 to 1.07 mm, average 1 mm from tip of tail; length of tail one-sixth to one-fifth of body length. Vulva situated 1.31 to 1.71 mm, average 1.57 mm, from anterior end dividing body in ratio of about 1:3; muscular vagina runs backwards; paired uterus extends 0.21 to 0.5 mm from anterior end and 0.71 to 1.3 mm from posterior end. Uterus filled with eggs and renders ovaries invisible by their out-stretch; eggs inside uterus near ovijector oval but slightly flattened on one side and measure 49 × 27 microns.

Host: Domestic pig.

Location: Stomach.

Locality: Bareilly, India.

Type specimen: Preserved in author's collection.

DISCUSSION

Syphacia spp. are as a rule parasites of rodents, except *S. obvelata* which may infect man and *S. bonnei* of the monkey *Alouata seniculus*. The present species is the first record of *Syphacia* from pigs, and this is the 5th species of *Syphacia* recorded from India. The 4 others are *S. sciuris* Mirza and Singh, 1935, recorded from a squirrel, *S. baylis* Mapplestone and Bhaduri, 1942, from a rat, *S. lahorea* Akhtar, 1953, from *Funumbulus pennanti argentescens,* and *S. tineri* Khera, 1954, from *Rattus norvegicus*. It is interesting to note that all *Syphacia* spp. have so far been recorded from the large intestine of their host, whereas the present species is found in the stomach of pig.

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The parasites were collected alive only from the stomach of the pigs freshly slaughtered in the pork market. On examination, the stomach contents did not show any trace of meat, offal, or pieces of tissue of any animal, which rules out the possibility of those pigs ingesting some rodent infected with *S. srivastavi*. If it is assumed that the ingested rodent was digested by that time and the parasites of the rodent continued to live in the stomach of the pigs, even then the pig may be considered as host of the parasite and stomach as the seat of predilection. In this case, however, pig appears to be the natural host of *S. srivastavi*.

Because male specimens were not available for comparison, the author had to depend solely upon the female characters for specific diagnosis. Chan (1951) has shown that the males of *S. obvelata* are very short-lived. They die shortly after fertilizing the females which live longer. This perhaps explains the absence of males in the author's collection.

Twenty-five species of *Syphacia* have been described so far. Of course some of them may prove to be only synonyms. The following table shows the measurements of only those species which are better comparable to *S. srivastavi*. The size of the egg is the most distinct character by which it differs more or less from all the species of *Syphacia* except *S. bonnei* Van Theil, 1926. But by the larger size of the worm, relatively long oesophagus, and the more complex structure of the anterior end, *S. bonnei* can be easily distinguished from *S. srivastavi*. *S. obubra* Baylis, 1936 appears to be almost similar to *S. srivastavi* in measurements, but the structure of their anterior ends differs to a considerable extent. The anterior end of *S. obubra* bears a remarkable dorsal cuticular frill which has 2 thickened horseshoe-shaped folds. The anterior end of *S. srivastavi* is very simple. This species differs from *S. montana* Yamaguti, 1943, distinctly by the smaller size of the egg and absence of teeth in the wall of the oesophagus. *S. srivastavi* can be differentiated from *S. muris* Yamaguti, 1935, by its larger size, relatively longer tail and smaller egg. *S. paraxeri* Sandground, 1933, and *S. nigeriana* Baylis, 1928, resemble *S. srivastavi* only in size. *S. paraxeri* can be distinguished from *S. srivastavi* by its comparatively shorter oesophagus, shorter tail, more anteriorly placed vulva and larger eggs. The present species differs from the genotype *S. obvelata* by its longer oesophagus and relatively distant vulva from the anterior end and much smaller egg.

The species has been named after Dr. H. D. Srivastava, M. Sc., D. Sc., Head of the Division of Parasitology, Indian Veterinary Research Institute, Izatnagar (U. P.), India.

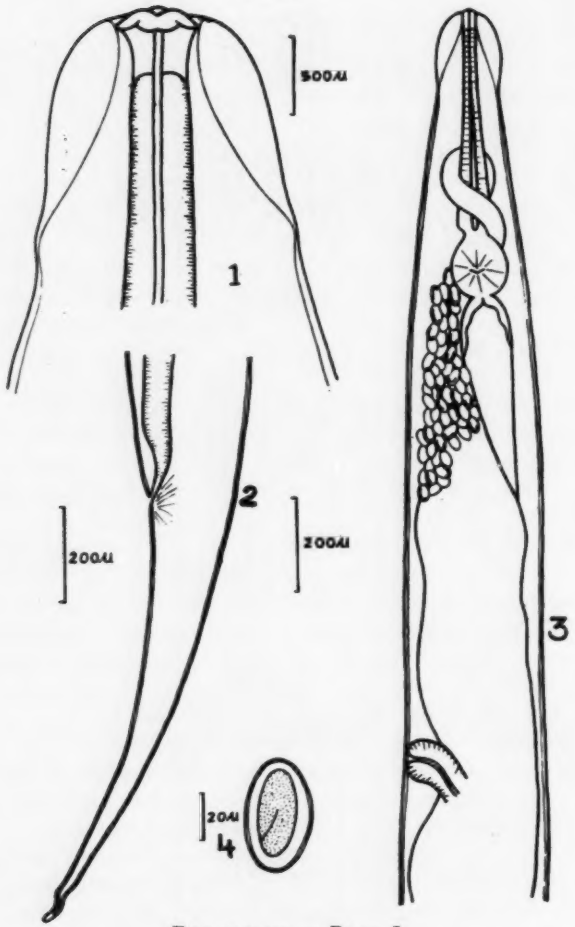
Reference

Chan, K. F. 1951 Life cycle studies of *Syphacia obvelata* and their relationship to chemotherapy. J. Parasit. **37** (Supp): 14.

TABLE I. *Measurements of some comparable species of* Syphacia. *All measurements are in millimeters except those for eggs, which are in microns.*

	S. obvelata	*S. paraxeri*	*S. nigeriana*	*S. muris*	*S. montana*	*S. bonnei*	*S. obubra*	*S. srivastavi*
Length	3.5 –5.7	4.6 –5.3	5.3	2.8 –3.4	3.7 –5.9	8.5	3.7 –5.2	5–5.7
Breadth	0.115–0.215	0.22–0.26	0.25–0.3	0.18–0.25	0.25–0.3	0.48	0.33–0.53	0.29–0.34
Oesophagus	0.305–0.4	0.33	0.35–0.42	0.25–0.29	0.3 –0.4	2.04	0.6 –0.75	0.57–0.71
Genital pore from ant. end	0.5 –0.74	0.62–0.82	0.7 –0.8	—	0.65–0.775	2.75	1.2 –2.1	1.31–1.71
Tail	0.515–0.705	0.59–0.7	0.85–0.95	0.32–0.35	0.45–0.9	1.48	0.5 –0.65	0.71–1.07
Egg	110–142 × 30–40	81–87 × 29–32	113–125 × 35–40	72–78 × 30–31	108–125 × 29–40	41.8 × 28.8	60–70 × 26–32	49 × 27
Host	Mice, rat and man	*Paraxerus palliatus*	*Taterillus gracilis*	Rat	*Microtus montebelli* *Clethrionomys smithi*	Monkey	Flying squirrel	Domestic pig

PLATE I



EXPLANATION OF PLATE I

Syphacia srivastavi n. sp.

FIGURE 1. Anterior end of female showing cephalic vesicle, lips, and pharynx.

FIGURE 2. Lateral view of the posterior end of female showing anus and the tail.

FIGURE 3. Lateral view of the anterior end of the female showing oesophagus, ovary, uterus, and vulva.

FIGURE 4. Embryonated egg from the ovijector.

THE TRYPANOCIDAL ACTIVITY OF NUCLEOCIDIN AGAINST *TRYPANOSOMA VIVAX* IN WEST AFRICAN ZEBU CATTLE

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During recent years a number of reports have been published on the activity of various antibiotics against trypanosomes. McCowan et al (1953) reported that *Trypanosoma equiperdum* failed to kill mice treated with erythromycin. Hewitt et al (1953) found that puromycin (achromycin) was effective against *T. equiperdum.* Puromycin was also shown to have a suppressive effect against *T. equiperdum, T. evansi, T. equinum, T. gambiense,* and *T. rhodesiense* (Tobie, 1954). Dr. R. S. Desowitz of this Institute (unpublished data) found that puromycin was curative when given subcutaneously to rats infected with *T. congolense* and a rat-adapted strain of *T. vivax.* Tobie and Highman (1956) reported that the aminonucleoside of puromycin was highly effective against *T. equinum, T. equiperdum, T. gambiense,* and *T. rhodesiense,* and somewhat active against *T. congolense.*

Thomas et al (1956) described a new antibiotic, nucleocidin, which was active against trypanosomes. The substance, which is produced by a new strain of *Streptomyces, S. calvus,* isolated from soil obtained in Dinepur, India, has been shown to be a glucoside of adenine in which the carbohydrate moiety of unusual structure is bound in ester linkage to sulfamic acid (Waller et al, 1957). Recently Tobie (1957) reported that nucleocidin was extremely active against 2 strains of *T. congolense* in mice and rats. Three daily doses of 0.05 mg per kg, a total of 0.15 mg per kg, produced approximately 95 percent cures in these rats and mice.

The following report gives the results of the activity of nucleocidin against *T. vivax,* transmitted by wild *Glossina morsitans,* in West African White Fulani zebu bulls.

The trial was arranged to take place at this Institute by the Chemotherapy Panel of the Tsetse Fly and Trypanosomiasis Committee of the Colonial Office, London.

MATERIALS AND METHODS

Cattle. Three White Fulani zebu bulls were purchased from the Federal Department of Veterinary Research, Vom, Northern Nigeria. All were vaccinated against rinderpest, bovine pleuropneumonia, and blackquarter (blackleg). They were classified for general physical condition as follows: 1 poor, 1 fair, and 1 good.

Drugs. A quantity of nucleocidin (lot No. 3653B—130A) was received from Cyanamid International, American Cyanamid Company, Pearl River, New York. Dr. H. J. Stutz recommended the dose rate and route of administration used.

Infection. Approximately 30 *Glossina morsitans* collected from the Mando Road fly belt (30 miles West of Kaduna) were fed on each of the cattle on 4 consecutive days. The infecting fly batches were rotated each day so that each animal was challenged by 90 different flies and by a total of 120 fly bites. Flies were maintained in Bruce boxes and were applied to the backs of cattle for the infecting feeds.

At the end of the challenge a proportion of the flies was dissected to determine the infection rate. Since Vom is in a tsetse-free area, there can be no doubt that these flies were the only source of infection.

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Treatment. The antibiotic was injected intramuscularly into the posterior crural region at a dose rate of 0.025 mg per kg as a 0.2 percent w/v solution in sterile distilled water. It was found that the drug did not go readily into solution; approximately an hour of shaking was required before all the crystals had dissolved.

Routine Examinations. The rectal temperature of each animal was taken daily between 7 and 9 a.m. Wet blood films were examined 6 times weekly for two and one-half months before challenging the animals, to ensure that they were not infected, and thereafter 6 times a week throughout the experiment. Each animal was weighed weekly during the entire course of the trial on an accurate cattle balance.

At 10- to 14-day intervals, the following blood examinations were made: (a) erythrocyte count, with the Thoma haemocytometer; (b) packed cell volume, with Wintrobe haematocrit tubes centrifuged at 3,000 revolutions per minute for 1 hour at a radius of 5.5 inches; (c) haemoglobin determinations with the Medical Research Council grey-wedge photometer.

In addition to the above tests, the serum was examined for glutamic oxaloacetic transaminase (S.G.O.T.) and glutamic pyruvic transaminase (S.G.P.T.) twice a week during the pre-patent, infected, and immediate post-treatment periods, employing the technique of King (1958). Clinical examinations were made daily by the authors.

Controls. No controls were included in this experiment, but experience has shown that *T. vivax* from the Mando Road area kills a healthy ox in about 2 months (Stephen, 1958).

Husbandry. The animals were housed in cattle sheds from sunset to sunrise. During the day they were kept in a small paddock and were provided with approximately 8 pounds of Atcha straw (*Digitaria exilis*), and 1.5 pounds of peanut cake per head per day. Rock salt (local "Kanwa") was provided ad libitum.

RESULTS

Infection. Trypanosomes were found in the peripheral blood of the 3 animals 12, 10, and 10 days, respectively, after the first fly feed. The tryanosome species was identified in Giemsa-stained thin films. Although no attempt was made to select a particular species of trypanosome for infecting these animals, all developed *T. vivax* infections.

The infection caused rapid loss of weight, a marked decline in erythrocyte counts, haemoglobin values, and packed cell volumes in each of the 3 cattle. The serum glutamic oxaloacetic transaminase values showed no marked variation from pre-infection values, but the serum glutamic pyruvic transaminase values rose sharply during the period of infection and dropped to pre-infection levels after treatment. They did not rise again during the relapse infections.

The parasitaemia, as determined by counting the number of trypanosomes in 60 fields of a wet blood film, employing a one-sixth inch objective and $\times 8$ eyepiece, is shown in table I.

Treatment. The infections were permitted to continue for 8, 10, and 10 days, respectively, before treatment was given. The dose volumes were as follows: ox 138, 1.4 ml; ox 98, 1.45 ml; and ox 102, 2.0 ml.

The 3 animals were all negative when examined on the following morning, 20 hours after treatment.

Post-treatment reactions. One hour after treatment the largest animal (ox 102), and thus the one receiving the largest amount of drug, was very drowsy and had stopped eating.

During the second hour the muzzles of all 3 animals were excessively moist, and grinding of the teeth was noticeable; all were salivating profusely, respiration rates were increased, and at times the animals were panting. The largest animal was unsteady on its feet.

At the end of the second hour 2 of the cattle were recumbent, but by the following morning they were all eating and appeared to be normal.

The temperatures dropped to within normal ranges 24 hours after treatment. Oxen 98 and 102 registered temperatures slightly below normal, i.e. below 100° F, on several occasions during the week after treatment.

Deaths. Ox 98 died 45 days after drug treatment. Autopsy revealed an acute fibrinous pericarditis with suffusions in the epicardium, particularly along the coronary grooves.

Relapses. All 3 animals relapsed to *T. vivax* in 33, 24, and 18 days after treatment. Trypanosomes were found sporadically in the peripheral blood and in small numbers during the first 2 months of the relapse infections. Although oxen 102 and 138 originally relapsed to *T. vivax,* subsequent thin films, stained by Giemsa, revealed the presence of *T. congolense* and the disappearance of the former organism. In the case of ox 138, *T. vivax* was found on 3 consecutive days following relapse, and then the blood was negative until the appearance of *T. congolense* 54 days later. *T. congolense* was identified in films from ox 102 sixty-four days after it first relapsed, but the exact time at which this organism appeared is not known. Trypanosomes were seen in wet films from this animal on 6 other occasions during this period, but they were always too scanty to be identified in stained preparations.

The relapse infections in the 2 surviving animals caused no serious harmful effects. Apart from a slight drop in weight and blood values during the 2 weeks after relapse, both animals continued to gain weight and to show improved blood values.

Other diseases. Seven days after treatment ox 138 developed a temperature of 104.2° F and examination of a Giemsa-stained thin blood film revealed moderate numbers of *Babesia bigemina* in the erythrocytes. The animal was treated with 6.75 ml of Phenamidine Isethionate, B. Vet. C.–4:4′–diamidinodiphenylether di-(β-hydroxyethane sulphonate)–in 40 percent solution, injected subcutaneously once only. This treatment did not cure the infection as piroplasms were seen again 25 days later, but the animal did not become clinically ill on this occasion.

Fly dissections. Thirty-five flies from the batch used to infect the cattle were dissected, and 9 (26 percent) were positive. Of these 9 flies, 6 were infected with *T. congolense* group and 3 with *T. vivax* group.

These results are summarized in table II.

DISCUSSION

T. vivax infections developed in the 3 animals in this experiment although dissections revealed a high percentage of *T. congolense* in the fly. Observations made during the course of other experiments at this Institute have shown that cattle infected by wild flies frequently develop an apparently pure *T. vivax* infection. However, if a relapse occurs after treatment, particularly with one of the phenanthridinium compounds, the relapse infection is often *T. congolense.* In this experiment both the pretreatment and the earlier relapse infections were due to *T. vivax.* Nucleocidin at 0.025 mg per kg appears to be less active against *T. vivax* than the phenanthridinium compounds in current use. The subsequent appearance of *T. congolense* in 2 animals showed that a latent infection with this species was present and that it was not cured. It is possible that a higher single dose or a course of treatments at the same dose would be curative.

Ox 138 did not relapse until 33 days after treatment, whereas oxen 102 and 98 relapsed in 18 and 24 days respectively. A possible explanation for this difference

TABLE I. *The parasitemia, as trypanosomes per 60 microscopic fields of wet blood film preparation, of White Fulani zebu bulls infected with* Trypanosoma vivax *transmitted by wild-caught* Glossina morsitans.

Date (1959)	4–9	4–10	4–11	4–12	4–13	4–14	4–15	4–16	4–17	4–18	4–19	⊕ 4–20	4–21	4–22
Ox 98	0	3	11	120	12	60	50	147	120	massive	250	120	0	0
Ox 138	0	0	0	3	58	60	24	93	50	28	10	122	0	0
Ox 102	0	2	1	5	48	180	83	82	120	34	128	90	0	0

⊕ Day treated

TABLE II. *Results of treatment of* Trypanosoma vivax *infections in West African cattle using nucleocidin at a dose rate of 0.025 mg per kg.*

Ox number	Condition at begining of experiment	Prepatent period	Infecting trypanosome	Period infected before treatment	Dose volume	Period treatment to relapse	Relapse trypanosomes	Number of days blood positive during post-treatment observation period	Post-treatment observation period	Remarks
138	Poor	12 days	*T. vivax*	8 days	1.40 ml	33 days	*T. vivax* *T. congolense*	6	95 days	Infected with *Babesia bigemina* 7 days after treatment for trypanosomiasis. Treated with phenamidine isethionate
98	Fair	10 days	*T. vivax*	10 days	1.45 ml	24 days	*T. vivax*	2	45 days	Died 45 days after treatment
102	Good	10 days	*T. vivax*	10 days	2.00 ml	18 days	*T. vivax* *T. congolense*	12	95 days	

is that ox 138 was treated with phenamidine isethionate for piroplasmosis 8 days after treatment with nucleocidin. Lourie and Yorke (1929) found that phenamidine and other aromatic diamidines possess considerable activity against trypanosomes. Thus the treatment for piroplasmosis may have delayed the appearance of the relapse organism in the peripheral blood.

The death of ox 98 forty-five days after treatment is not suspected to be due to drug toxicity. This animal was classified as fair at the beginning of the experiment, and had not progressed satisfactorily during the preinfection period. The exact cause of death is not known.

There is no doubt that the initial infections developed by these cattle were acute. Body weights and all haematological values showed a sharp drop during the time that the infection was permitted to run.

The rise in serum glutamic pyruvic transaminase during the parasitaemic period is of considerable interest. As the S.G.O.T. and S.G.P.T. have been shown to be raised in necrotic processes in the body, probably as a result of cellular damage, it was thought that they might give a useful indication of the occurrence of drug toxicity.

In man, S.G.O.T. and S.G.P.T. values have been used as an aid to diagnosis and differential diagnosis of cardiac and hepatic conditions. (Pryse-Davies and Wilkinson, 1958, and Henry, 1959).

Cornelius et al (1959) failed to demonstrate significant G.P.T. levels in either normal bovine liver or in the sera of cattle suffering from severe liver damage induced by carbon tetrachloride poisoning. It is possible that the progressive rise of S.G.P.T. found during the parasitaemic period in these 3 cattle had a different origin. The S.G.P.T. levels fell to normal in all 3 animals immediately after treatment; that is, at the time when the trypanosomes disappeared from the peripheral blood. We have found high levels of both G.O.T. and G.P.T. in homogenates and suspensions of living trypanosomes. However, the highest S.G.P.T. level in this experiment was not found in the animal which had the highest parasitaemia. Lippi and Sebastiani (1958) found a progressive rise in the serum level of G.O.T. and G.P.T. during the course of experimental *T. brucei* infections in guinea pigs. They attributed these findings to increasing lesions of the liver, myocardium, adrenal glands, and nervous tissue. The reasons for the rise of this enzyme, in trypanosome infections, are being investigated by one of us (A.R.G.).

SUMMARY

The antibiotic, nucleocidin, appears to possess considerable activity against *T. vivax* infections in West African White Fulani zebu cattle. A single intramuscular injection at a dose rate of 0.025 mg per kg cleared the peripheral blood of trypanosomes within 20 hours.

The infection was not cured, however, and relapses occurred 18, 24, and 33 days after treatment in the 3 animals used in the experiment.

ACKNOWLEDGMENTS

We are indebted to Dr. K. C. Willett, Director of the West African Institute for Trypanosomiasis Research, for his interest in this work, and for critically reading the manuscript. We also wish to thank our African technical assistants and herdsmen for their diligence during the experiment.

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ANNOUNCEMENT

HELMINTHOLOGICAL SOCIETY OF WASHINGTON OBSERVES FIFTIETH ANNIVERSARY

Saturday, October 8, 1960, the Helminthological Society of Washington will observe its Fiftieth Anniversary. It is hoped that all persons who are interested in parasitology and the related sciences will keep this date in mind and arrange to attend. The scientific program will be held at the University of Maryland, College Park, Maryland. A banquet will be held during the evening at the Naval Officers Club at Bethesda, Maryland. Dr. Chauncey D. Leake, President of the American Association for the Advancement of Science, will be speaker of the evening.

HAEMOGREGARINES IN SNAKES: THE INCIDENCE AND IDENTITY OF THE ERYTHROCYTIC STAGES*

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The traditional methods of displaying reptile specimens in zoos provide ideal conditions for transmission of protozoan parasites due to the humid cage atmosphere and the sand or leaf mulch substrates used to simulate natural habitats. These physical conditions favor arthropod reproduction; the concentration of potential hosts favors transmission of whatever parasites may be present. Indeed, the mortality rates typical of zoo reptile collections substantiate the probability that more than just the nutritional and "psychological" effects of confinement and display affect the longevity of the captive population. Only impractically elaborate precautions could eliminate the possibility of parasite transmission under the display conditions deemed necessary to assuage the aesthetic sensibilities of the general public. Therefore, examination of zoo specimens for parasites may be expected to be quite rewarding; seldom are conditions contributing to high parasite concentrations combined with the ready availability of hosts from all over the world that a zoo offers.

This report presents data concerning the incidence and identity in captive snakes of the protozoan blood parasite generally included in the genus *Haemogregarina*. These data were gathered during 1955 through 1958 as part of a program concerning experimental transmission of reptilian blood parasites. The authors are grateful to the curators of the reptile collections at the Lincoln Park Zoo, Chicago, Illinois, the Toledo Zoo, Toledo, Ohio, the Cleveland Municipal Zoo, Cleveland, Ohio, and to the zoo personnel at these institutions for their physical aid and moral support in the pursuit of the specimens from which blood and these data are taken.

MATERIALS AND METHODS

Thin blood films on standard 1- by 3-inch microscope slides were made from each snake in the aforementioned zoos. The blood films were air dried, fixed in absolute methyl alcohol and stained with Giemsa stain (Harleco) at pH 7.0, using Sorensen M/30 phosphate buffer as stain diluent in a ratio of 1 part stain to 20 parts buffer.

Blood was taken by puncture of a superficial vein in the roof of a snake's mouth rather than by the usual tail clipping, so as to avoid mutilating these display specimens. This procedure was chosen because snake jaw articulation is such that opening the mouth to 180 degrees is painless to the snake and easily accomplished. The invisible (and temporary) wound in the snake's mouth is much more acceptable to zoo personnel than permanently shortened snakes. Saliva is virtually absent in the roof of the snake's mouth, although when dealing with venomous specimens, the venom is troublesome (particularly haemolytic venoms) if allowed to accumulate in the mouth before venipuncture is accomplished. No effects detrimental to the snakes were

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apparent from this blood-letting procedure, even though sterile precautions were not observed

In addition to the blood specimens taken from snakes that had been resident in the zoos for 3 months to as long as 4 years, blood was examined from snakes immediately after their capture in central and southwestern United States, and from snakes kept in the laboratory for transmission studies.

Selected blood samples were fixed in Schaudinn's fluid and stained by Heidenhains iron-hematoxylin method, subjected to the Fuelgen nuclear reaction, or stained with toluidin O, as well as held for various periods of time (10 minutes to 8 hours) to observe the morphology of the living parasites.

The measurements indicated in table II were made using either a Spencer filar ocular or a calibrated reticle in a 10× ocular and the 97× oil-immersion objective. Each measurement was made in bright field and corroborated by observation and measurement under phase contrast. Morphological detail in the Giemsa stained parasites, particularly cytoplasmic vacuoles or granules, is much easier to see under phase contrast. The rationale of the analysis of the numerical data will be discussed below.

Measurements stated are in microns.

RESULTS AND DISCUSSION

Table I presents a summary of the incidence of haemogregarines in the snake species examined from the 3 zoos surveyed and the levels of infection encountered. The parasitaemias (number of infected erythrocytes per 100 erythrocytes) are based upon counts of 1000 erythrocytes from each specimen. Parasitaemias indicated as 0.1 percent showed 10 or less parasites per 1000 cells. The highest parasitaemia (82.4 percent) was seen in a Marcy's garter snake (*Thamnophis marcianus*). Blood from this specimen was examined repeatedly over a period of 3 months during which time the snake appeared completely normal; it died quite suddenly, apparently from a bacterial infection in the lung.

TABLE I. *Rate of infection of captive snake specimens with intra-erythrocytic stages of haemogregarine parasites. (All snakes included were resident in the zoos for at least 3 months.)*

Host genus	Common name of species	No. examined	No. infected	Parasitaemia range (pct)
Python	reticulated python, carpet python, ball python, Indian python	23	5	0.5 to 7.8
Constructor	boa, rubber boa, South American boa	15	6	0.1 to 3.1
Pituophis	pine snake, bull snake	36	9	0.1 to 0.4
Elaphe	pilot black snake, yellow rat snake, Korean rat snake, corn snake, Lindheimer's rat snake	51	18	0.1 to 5.0
Lampropeltis	speckled king snake, common milk snake, Mexican milk snake, common king snake, chain king snake	77	45	0.1 to 7.5
Oxybelus *Dryophis* *Leptophis*	common vine snake, green vine snake	14	8	0.1 to 23.2
Masticophis	coachwhip snake	105	17	0.1 to 0.5
Thamnophis	common garter snake, Marcy's garter snake	66	5	0.1 to 82.4
Natrix	common water snake, tigrine water snake	29	8	0.1 to 0.7
Crotalus	prairie rattlesnake	15	0	—
Ancistrodon	water moccasin	39	33	0.1 to 33.8
	TOTAL	600	154	
	INFECTION RATE	30.8 pct		

The initial impression that the intra-erythrocytic stage of the parasite represented a single morphological entity was dispelled by critical observation and measurement, so that at least 4 distinct types of parasite morphology were identified. The relative density of any one such type depends upon the state of infection (i.e., recent infection or one of long standing), as is verified by data taken from snakes showing daily variations in parasitaemia (see fig. 11 and below). Arbitrary numbers from 1 to 4 were assigned each of these intra-erythrocytic types and differentia-

tion of the types was based upon cytological as well as dimensional criteria; identifying criteria are as follows:

Type 1 (Figs. 1 and 5). Oval, slightly curved with rounded ends; nucleus

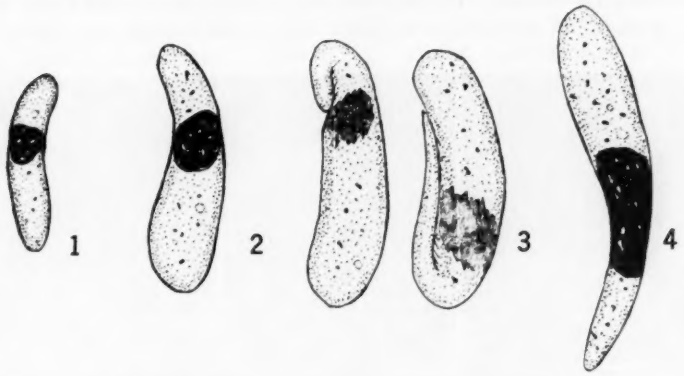

FIGURE 1. "Type 1" parasite.
FIGURE 2. "Type 2" parasite.
FIGURE 3. "Type 3" parasite, representing the two extreme (quarter-tail and full-tail) morphologies included in this category.
FIGURE 4. "Type 4" parasite.

round or oval band, centrally located, with dense clumped basichromatin and frequently incomplete nuclear fragmentation; cytoplasm denser at the periphery, containing few granules or vacuoles; host cell normal, no enlargement or nuclear displacement. Dimensions: length 10.5 ± 1.58, range 5.4 to 14.4; width 3.3 ± 0.63, range 1.3 to 5.9, rate of occurrence 3 to 64 per 10,000 cells.

Type 2 (Figs. 2 and 6). Oval, slightly curved, with smoothly rounded ends; nucleus round to slightly oval, as wide as the body, often eccentrically located; chromatin in thick strands or clumps; cytoplasm finely stippled or filamentous, distinct red, blue or birefringent granules common; host cell intact, nucleus laterally displaced, no hemolysis. Dimensions: length 13.6 ± 1.19, range 9.0 to 18.2; width 3.75 ± 0.48, range 1.8 to 6.5; rate of occurrence 5 to 666 per 10,000 cells.

Type 3 (Figs. 3, 8, 9). Oval in outline in situ, elongate, bluntly rounded on one end, attenuated on other end when removed from the erythrocyte; nucleus small and dense, showing a tufted membrane, and usually located in the area of curvature of the primary segment; cytoplasm finely stippled in "long-tailed" forms and distinctly granular in "short-tailed" forms; host cell intact, but slightly enlarged in some hosts, nucleus laterally displaced, no hemolysis. Dimensions: length 12.8 ± 0.93, range 7.2 to 16.2; width 4.20 ± 0.51, range 2.7 to 7.3; rate of occurrence, 5 to 85 per 10,000 cells.

Type 4 (Figs. 4 and 10). Elongate, slender and tapered when extracellular; ovoid, in an apparent capsule when intracellular; nucleus irregularly round to oval band, basichromatin in dense granules, eccentrically located; cytoplasm finely granular with larger red or blue granules irregularly distributed; host cell normal size or slightly "stretched" on long axis, nucleus laterally displaced, no hemolysis or loss of hemoglobin even after the parasite leaves the cell. Dimensions: length 15.8 ± 1.37, range 10.8 to 21.2; width 2.60 ± 0.28, range 1.9 to 3.3; rate of occur-

rence, 4 to 53 per 10,000 cells or in exceptional cases constitute more than 50 percent of the parasites present.

Normal, mature, non-parasitized erythrocytes (100 per snake species) were measured and the haemoglobin distribution in the cytoplasm and shape, position and configuration of the nucleus recorded. These data appear in column one of

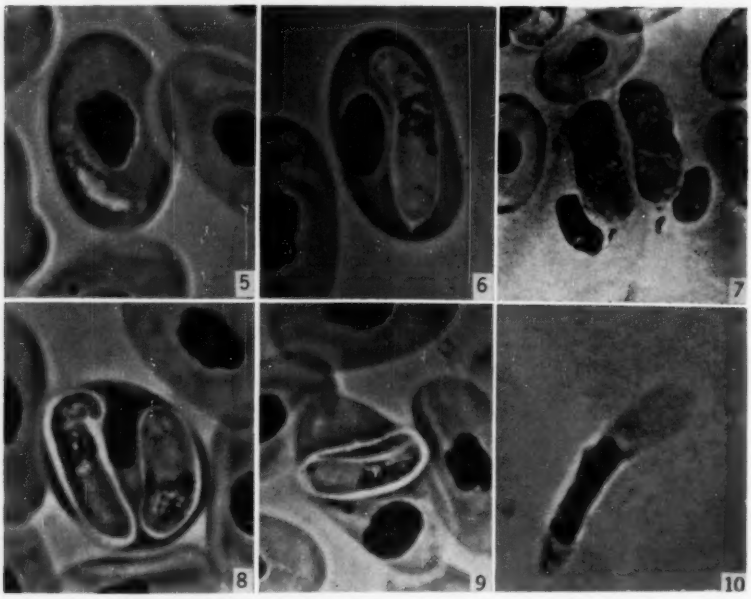

FIGURE 5. "Type 1" parasite; from *Thamnophis marcianus,* Giemsa stain, dark phase contrast, × 2000.

FIGURE 6. "Type 2" parasite; from *Boa hortulana cooki,* Giemsa stain, dark phase contrast, × 2000.

FIGURE 7. "Enlarged" parasite; from *Elaphe obsoleta lindheimeri,* Toluidin O stain, dark phase contrast, × 1820.

FIGURE 8. Two "Type 3" parasites in the same host cell; from *Lampropeltis getulus floridanus,* Feulgen nucleal reaction, dark phase contrast, × 2000.

FIGURE 9. "Type 3" parasite; from *Python spilotes,* Giemsa stain, dark phase contrast, × 2000. Note the polar displacement of the erythrocyte nucleus and erythrocyte cell wall distortion caused by the parasite.

FIGURE 10. "Type 4" parasite; from *Boa hortulana cooki,* Giemsa stain, dark phase contrast, × 2000. The parasite has just broken out of the erythrocyte partly visible in the lower left corner of the figure.

table II and represent the normal morphology of the non-parasitized erythrocyte. Survey of 10,000 erythrocytes from each of these snake specimens provided the remainder of the data shown in table II. Examination of such a large number of cells per snake host was necessary to assure that the parasite measurements taken formed a statistically valid sample of the parasite population even though the parasitaemias were below 1.0 percent in some instances.

Causal comparison of these data implies that the dimensional differences between the parasites found in one species of snake and those found in any other snake species are rather consistently large. A method of critically assessing in what

manner dimensional variation of the parasites between species of host snakes is related to the dimensions of the normal, non-parasitized erythrocyte (since the erythrocyte size also varies from one snake species to another) is provided by the product moment correlation coefficient. This analytical procedure yields a numerical index of the degree of co-variance of two measurements in the same (or intimately related) populations. Since the correlation coefficients show a normal distribution in tests where zero correlation is assumed (null hypothesis), the significance of an apparent correlation can be determined directly from probability tables (Fisher, 1948). Applying this method of analysis to the type 2-parasite, normal-erythrocyte populations yields a correlation coefficient of 0.0164 using the linear length dimension of each entity as the criterion or 0.0523 using the area of the elliptical plane described by each entity (assuming that the ellipse is regular in both cases) as the criterion. Similarly, the correlation coefficients using the same criteria for the type 3 parasites are 0.0203 and 0.0671 respectively. Each of these correlation statistics exceeds the 5 percent level of significance and therefore the null hypothesis cannot be rejected; the parasite sizes as shown in table II are not significantly correlated to the size of the non-infected erythrocytes typical of the snake species harboring the parasite.

Since erythrocyte size typical of the host snake species apparently does not control the parasite size, it is essential to determine whether the parasites as described by dimension represent distinct entities or merely various samples from

TABLE II. *Comparison of the dimensions (in microns) of the intra-erythrocytic haemogregarine parasites in 10 species (or subspecies) of snakes as related to the noninfected erythrocyte size. The figures appearing under the mean dimensions of each stage parasite indicate the percent of the infected erythrocytes that were greater than 4 standard deviations larger than the mean of the non-infected erythrocyte (i.e., 1 standard deviation larger than the non-infected erythrocyte).*

Snake species	Non-parasitized erythrocyte				Parasite Dimensions							
					Type 1		Type 2		Type 3		Type 4	
	Mean	S.D.	Ratio 1/w	Area	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Lampropeltis getulus getulus	18.7 ×10.3	2.25 0.84	1.81	606			13.2 ×3.2 24	0.72 0.38			13.3 ×2.7 25	0.94 0.65
Boa hortulana cooki	18.6 ×10.3	0.91 0.71	1.80	602	10.9 ×3.1 0	1.53	13.0 ×3.7 40	1.13 0.70	12.7 ×3.9 24	0.76 0.41	18.3 ×3.3 37	1.80 0.33
Thamnophis marcianus	17.5 ×9.8	1.00 0.68	1.79	539	10.0 ×3.4 24	1.44 1.36	14.5 ×3.9 28	1.22 0.77	14.8 ×3.9 40	1.39 0.42	14.0 ×2.2 31	1.27 0.60
Coluber sp.	16.9 ×9.7	1.44 0.81	1.74	515	10.9 ×3.7 6	1.96 0.52	14.9 ×4.2 18	1.15 0.46	14.9 ×3.9 19	0.98 0.39	16.8 ×2.3 61	0.02 0.11
Lampropeltis getulus holbrooki	17.2 ×9.9	1.38 0.92	1.74	535	9.4 ×3.2 24	0.36 0.18	12.4 ×3.9 40	1.25 0.49	12.2 ×4.1 24	1.37 0.69	10.8 ×2.7 37	1.56 0.70
Python spilotes	16.4 ×9.8	1.38 0.78	1.67	505			14.9 ×4.3 94	0.87 0.58	14.4 ×4.4 95	1.12 0.59		
Elaphe obsoleta	16.5 ×10.1	1.32 0.82	1.63	524			14.5 ×3.3 77	1.36 0.68	11.7 ×3.9 56	1.01 0.26	13.7 ×1.9 100	3.52 0.22
Lampropeltis getulus floridanus	16.8 ×10.3	1.03 0.85	1.63	544			13.8 ×3.5 80	1.45 0.51	10.6 ×4.1 82	0.87 0.78	17.0 ×2.4 72	1.98 0.57
Masticophis flagellum	14.7 ×11.2	1.17 0.87	1.31	520			12.3 ×4.1 85	1.23 0.52	12.6 ×4.5 99	1.88 0.77	10.7 ×3.5 91	0.06 0.01
Natrix sipedon	16.2 ×12.7	1.23 1.27	1.23	647	11.6 ×2.9 45	2.80 0.74	14.2 ×3.4 49	0.88 0.29			14.5 ×2.5 40	1.48 0.64

the same population of parasites. Stated more biologically, this question asks if all these parasites are the same species regardless of host snake species, assuming linear dimension of the parasite to be a species criterion. The statistic useful in this regard is *t*, the probability of obtaining 2 different mean dimensions and standard deviations from finite samples of the same population. Comparing the mean length of each type 2 parasite to the mean length of every other type 2 parasite listed in table II is readily accomplished for the 45 possible comparisons and is weighted by the standard deviation of each mean and size of each sample in the following manner:

(1) Standard error of mean $(\text{S.E.}) = \frac{\text{S.D.}}{\sqrt{n}}$

(2) Standard error of mean difference $(\text{S.E.D.}) = \sqrt{(\text{S.E.}_1)^2 + (\text{S.E.}_2)^2}$

(3) $$t = \frac{m_1 - m_2}{\text{S.E.D.}}$$

where m_1 and m_2 are the means of the respective populations. At the 5 percent significance level, 23 pairs are not sufficiently different from each other to indicate that they are samples from discrete populations; 22 pairs are sufficiently distinct at the 5 percent level to indicate that they are samples from discrete populations, using either length or plane elliptical area as the criterion. In these comparisons, n (see equation (1) above) varies between 19 (*Python spilotes*) and 666 (*Thamnophis marcianus*). The parasites in erythrocytes of *Lampropeltis getulus floridanus* are dimensionally indistinguishable from all the other parasites listed. Obviously, this results from the closeness of the mean of these parasites to the mean dimension of all type 2 parasites observed; this latter mean is 13.7, standard deviation equals 0.91 where n equals 1144. *Masticophis flagellum* parasites are indistinguishable from parasites in all other snake species observed except *Thamnophis marcianus, Coluber* sp. and *Elaphe obsoleta*. Parasites from the remaining snake species are identical to parasites from at least 2 (usually 3 or 4) other host

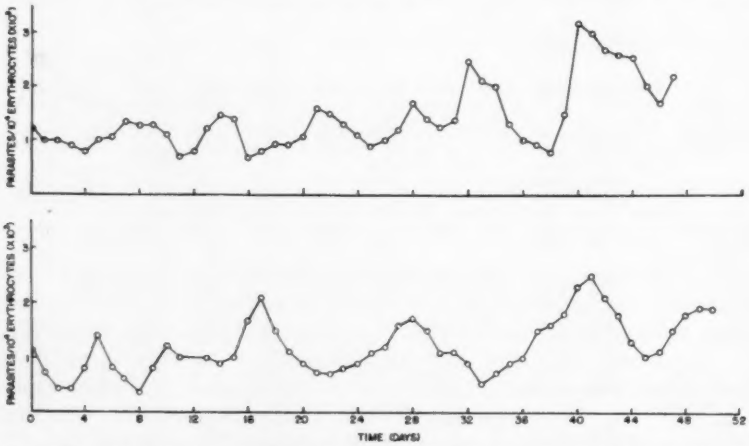

FIGURE 11. Variations in parasitaemia levels with time in a water snake, *Natrix sipedon* (upper curve) and a rat snake, *Elaphe obsoleta* (lower curve). These parasitaemia patterns are typical of the 17 specimens followed by daily blood examination.

species. Similar comparison of the width of each parasite to the same dimension of every other parasite of the same stage in all 10 species or subspecies of snakes indicates that the parasites are all members of the same population at the 1 percent significance level.

An additional feature to be noted in table II is that in 4 of the snake species observed, the majority of the erythrocytes infected showed enlargement greater than 1 standard deviation beyond the dimensions typical of the non-infected erythrocytes of that species of snake. These data are represented in table II by the percentage figures appearing under each parasite mean length. Again, correlation between type 2 parasites and normal erythrocytes in these 4 species was not significant at the 5 percent level, indicating that erythrocyte enlargement probably does not result from mechanical distortion of the red cell by the parasite.

Variation in the level of parasitaemia in single specimens examined daily is exemplified by figure 11. The variability shown in figure 11 is typical of the 17 specimens examined in this fashion for as long as 60 days. It is significant that the variations in parasitaemia show both increase and decrease from day to day, indicating that although the red cell life span in reptiles is quite long, infected erythrocytes are removed from the circulating blood and previously uninfected cells are being invaded by the parasite.

CONCLUSIONS

In no instance could distinction of sexually dimorphic intra-erythrocytic forms of the parasites be made consistently in the same host specimen. Figure 7 illustrates the morphology of intracellular forms found in 1 rat snake (*Elaphe obsoleta lindheimeri*) which was also infected with the microfilaria, *Macdonaldius seetae* Khanna, (Hull and Camin, 1959). This large form was also found in a few other hosts, indicating that the nematode was not the cause of the enlargement. Whenever such forms were found, they were always associated with lysed or lysing erythrocytes or with haemopoetic cells in the bone marrow or spleen. Only when conditions which fostered lysis of the red cells were used, such as suspending the snake blood in slightly hypotonic media before smearing or preparing thick films wherein the blood dried slowly on the slide (thus promoting hypertonic conditions before fixation by dessication occurred), could such forms be found. The relative infrequency of these enlarged forms in properly handled blood specimens in comparison to the frequency of type 3 forms indicates that such enlargement results from lysis of the red cell to release the immature parasite, assuming that the type sequence of 1 through 4 described above represents progressive stages in the maturation of the intra-erythrocytic parasite. In no instance were these large, lysed-cell forms encountered in ratios to type 4 parasites that would indicate a macrogametocyte-microgametocyte (anisogamous) system. It is concluded that sexual dimorphism is not a characteristic of these parasites in the snake host.

The 2 haemograms (fig. 11) are typical of the two general host-parasite relationships observed among the 17 specimens examined daily for parasitaemia levels. Peak parasitaemias occurred at 6 to 8 day intervals when the parasite load in the circulating blood exceeded 500 parasites per 10,000 erythrocytes (8 cases) and at 12 to 14 day intervals when the parasite load was below 500 parasites per 10,000 erythrocytes (9 cases). If this periodicity is an indication of the schizo

gonic generation time, one can explain the 7-day versus 13- to 14-day cycling of the parasitaemia levels by either, (1) assuming that two discrete but relatively synchronous populations are reproducing 180 degrees out of phase with each other in the more highly parasitized specimens while only 1 such population appears in the case of the lower parasitaemias or, (2) 2 species of haemogregarines showing different schizogonic generation times were observed. The first hypothesis seems more tenable at present because of the very real possibility that all or most of the snake haemogregarines are members of a single species, or at best, strains of a few species.

Survey of the literature pertinent to snake haemogregarines indicates that there are at least 24 named species (Dobell, 1908) in the genus *Haemogregarina*. The majority of these species names have been erected solely on the basis of the morphology of the intra-erythrocytic form of the parasite; frequently, the species name of the parasite identifies the genus or species of the single host in which it was found. Some authors (Roudabush and Coatney, 1937; Dobell, 1908) recognized the danger inherent in such a practice as long ago as 1908, but no taxonomic analysis of this virtual plethora of *Haemogregarina* species can be made until more information concerning the life cycle of these parasites is available.

The wide variety of snake hosts from which the above data are taken demonstrates that there is an extreme degree of morphological similarity among the intra-erythrocytic forms of these parasites, regardless of the species or genus of the snake host. The foregoing statistical comparisons of dimensional characteristics of the parasites when they occur in different host genera or species indicates the probable species identity of all the parasites encountered in this study. Such statistical comparison of course does not prove this identity since statistical procedure cannot be taken as ipso facto proof of any comparison, but it does indicate that the probability of species identity is well within the 5 percent level of significance that is commonly held to be adequate in biological measurement.

A further factor in the stability of existing species names for snake haemogregarines is the mounting evidence that these parasites can be transmitted from one genus of snake to another. Also, since it is possible that many of the parasites now included in the genus *Haemogregarina*, if the normal host is a snake, are truly members of genus *Hepatozoon* (see Ball, 1958), the taxonomic position of these parasites is completely in doubt until further details of the life cycle of the parasites in the snake hosts and/or their vectors are determined. It is particularly important to note that diagnosis of genus *Haemogregarina* as it now stands specifies the occurrence of reproductive activity of the parasites in the erythrocytes (Grasse, 1952), which situation does not obtain in the parasites described in this report. In view of all this, it is our conclusion that the parasites treated in this report should be considered members of genus *Hepatozoon*. Further, all morphological and cytological characteristics of the parasites discussed herein indicate that they are members of a single species. This conclusion is further supported by the statistical comparison of the dimensional characteristics of the parasites in a wide variety of host snakes. No species name is proposed at this time in order to avoid further confusing the taxonomy of these and allied protozoan hematozoa.

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RESEARCH NOTES

VIRUS-LIKE PARTICLES IN AN *ENTAMOEBA HISTOLYTICA* TROPHOZOITE.

During an electron microscopy study of the nuclear components of *Entamoeba histolytica* small orderly arranged particles were observed in a section of a trophozoite. The particles were grouped in 3 rows near the nucleus (fig. 1). Although several hundred sections of amebae were examined, only the section illustrated contained the particles. A few appear slightly ovoid and the remainder are spherical and measure approximately 400 A in diameter. Each particle exhibits a well-defined limiting membrane and a finely granular matrix which contains 1 or more electron dense bodies. The size and structure are compatible with sectioned virus particles as seen in electron microscopy.

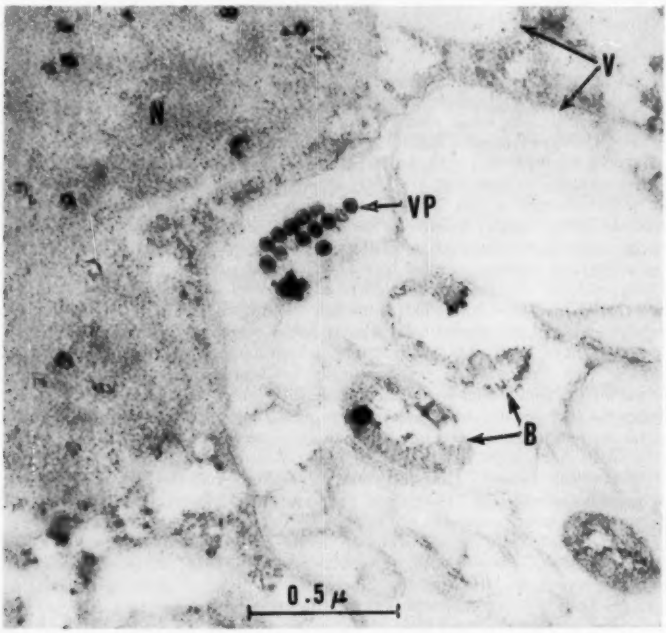

FIGURE 1. A section of an *Entamoeba histolytica* trophozoite fixed with chromium. The cytoplasm contains many food vacuoles (V) some of which may contain bacteria (B) undergoing digestion. The nucleus (N) may be irregular in electron micrographs and is more densely granular than the cytoplasm. Virus-like particles (VP) are aligned in three rows near the nucleus.

The trophozoite which contained the particles was of the NRS strain. This strain was isolated originally from *Macacus sinicus* in 1929 by Dobell (1931, Parasitology **23:** 1–72). It has been maintained in this laboratory for over 10 years on egg slants with a buffered saline overlay and rice starch. A mixed bacterial flora was present. Trophozoites from a 48-hour culture were fixed 15 minutes at 0 to 5 C in a solution containing 3 percent chromic acid, 4 percent formalin and 0.85 percent sodium chloride as recommended by Low and Freeman (1956, J. Biophysic. and Biochem. Cytol. **2:** 629–632). Dehydration was accomplished in a graded series of methyl alcohol and the trophozoites were embedded in a 3:1 mixture of *n*-butyl and methyl methacrylate polymerized at 40 to 50 C. Thin sections were cut on a Porter-Blum microtome, mounted on formvar-covered grids, and examined with an R.C.A. electron microscope, E.M.U.-3 at 100 KV.

Deutch and Zaman (1959, Exp. Cell Res. **17** (2): 310–319) have described electron dense particles in *Entamoeba invadens* which they interpret as chromatoid material. They pointed out the striking resemblance of the material to some virus particles. In contrast, the particles which we observed in *E. histolytica* are more discrete and twice the size of the objects found in *E. invadens*. Also, the particles are rare in *E. histolytica* whereas the objects in *E. invadens* apparently are uniformly present. At high magnifications the bodies found in *E. histolytica* do not exhibit a crystalline lattice structure.

(Supported in part by grants E-2347 and H-2549, National Institutes of Health, U.S. Public Health Service).—J. H. MILLER AND J. C. SWARTZWELDER, *Louisiana State University School of Medicine, New Orleans.*

A CESTODE LIFE CYCLE INVOLVING LAND SNAILS AND SHREWS.

Five species of land snails have been found to harbor cysticercoids of the tapeworm *Liga soricis* Neiland, 1953. The adult cestode has been recovered from the small intestine of the wandering shrew, *Sorex vagrans* Baird, by the author, and from *S. bendirii* Merriam and *S. obscurus* Merriam by Neiland (1953, J. Parasit. **39:** 487–494). Species of snails that have been found infected with this cysticercoid are *Haplotrema sportella* Gould, *Haplotrema vancouverensi* Lea, *Vespericola columbiana* Lea, *Triodopsis germana* Gould, and *Allogona townsendiana* Lea. The cysticercoids are generally located in the surface tissues of the kidney or the mantle. They may, however, occur in other soft parts of the snail.

Shrews apparently become infected by eating infected snails. The stomach from one shrew contained fragments of the shell and radula of *Haplotrema* sp., and 11 recently excysted cysticercoids were recovered from the small intestine. The cysticercoid resembles the adult worm, with the same number of morphologically similar hooks on the scolex.

Cysticercoids have been fed to laboratory white mice, white rats, golden hamsters, and chickens. Only two recoveries of experimentally fed tapeworm cysts have been made. Both recoveries were from the golden hamster, one after 18 hours and the other after 60 hours. In both instances the tapeworms had not matured enough to develop proglottids. Tapeworms were not recovered in experimental infections that had gone over 60 hours.

Infected snails have been collected from Coos, Benton, and Lane counties in Oregon and from Gray's Harbor, Mason, Thurston, and Wakiakum counties in Washington. Infected shrews have been collected by the author in Coos and Benton counties and by Neiland in Clackamas and Multonomah counties in Oregon.

The percent of infections by cysticercoids based on a total of 452 snails examined is as follows: *V. columbiana,* 16 percent; *H. vancouverense,* 4 percent; *A. townsendiana,* 3.7 percent; *T. germana,* 3.2 percent; and *H. sportella,* 2 percent.

Further research is being conducted on the life cycle with respect to distribution, incidence, mode of infection, and development time of the cysticercoid in the snails. In addition, attempts are being made to secure sexually mature worms from experimental animals.

The work was carried out in the laboratory of Dr. Ivan Pratt and was supported in part by a Science Faculty Fellowship from the National Science Foundation and by U.S.P.H.S. Grant E 867CC3.

(Published with the approval of the Oregon State College Monographs Committee Research Paper No. 375, Department of Zoology, School of Science.)—ALBERT G. CANARIS, *Zoology Department, Oregon State College, Corvallis.*

THE STATUS OF THE GENERA *ACANTHATRIUM* FAUST, 1919, AND *PROSTHODENDRIUM* DOLLFUS, 1931 (TREMATODA: LECITHODENDRIIDAE)

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In a recent paper, Etges (1960) reviewed briefly the lecithodendriid genera, *Acanthatrium* Faust, 1919, and *Prosthodendrium* Dollfus, 1931. In this paper the suppression of *Acanthatrium* as a generic synonym of *Prosthodendrium,* as stated by Yamaguti (1958), and the use of both names as subgenera in the revised genus *Prosthodendrium* were accepted. Yamaguti referred to Dubois' (1955) revision of "subgenera" (note plural) in a footnote (op. cit., p. 821). Further study by the writer reveals that Dubois (1955) treated only members of the subgenus *Prosthodendrium* and did not revise the subgenus *Acanthatrium.* Moreover, the suppression of *Acanthatrium* Faust, 1919, in favor of *Prosthodendrium* Dollfus, 1931, (apparently by Yamaguti) has violated the law of priority. The present note is intended to clarify the systematics of the *Acanthatrium-Prosthodendrium* complex.

The reader is referred to the discussion by Etges (1960) supporting the unigeneric concept of this complex. Comparisons of life histories and morphology of adult worms show the subgenera *Acanthatrium* Skarbilovich, 1948, and *Prosthodendrium* Dollfus, 1937, to be congeneric. The current basis for separating these subgenera is the presence (*Acanthatrium*) or absence (*Prosthodendrium*) of spines on the wall of the genital atrium; however, as will be pointed out below, this feature is not clear-cut and definite, but forms a graded series.

The illustrations below depict the graded sequence of genital atrium and atrial spine development in selected members of these subgenera. Phylogenetic significance of this sequence is not intended by the writer. Figure 1 shows the minute, unarmed form of genital atrium found in *Prosthodendrium swansoni* Macy, 1936; in fact, a definite atrium is not mentioned by Macy (1936) in his diagnosis of *P. swansoni.* Figure 2 shows the small atrium of *P. naviculum* Macy, 1936, in which minute "papillae" are found; this condition is seen to be intermediate between "typical" *Prosthodendrium* and *Acanthatrium* species. Figure 3 shows the larger atrium of *Acanthatrium oligacanthum* Cheng, 1957, armed with only 9 minute spines, 0.0026 mm long. Figures 4 through 8 indicate gradual enlargement and complication of the genital atrium, and increase in the number and length of atrial spines, culminating in the elaborate structure seen in *A. alicatai* Macy, 1940 (fig. 8), which has "numerous" broad spines 0.026 mm long, and the atrium divided into 3 distinct muscular pockets. This gradual sequence further indicates the difficulty of clearly defining these 2 groups on a generic level.

Evidence presented and cited above indicates Yamaguti (1958) to be correct in placing all members of this complex in one genus. Yet, since most members of the group clearly lack or possess atrial spines, the subgenera *Acanthatrium* and *Prosthodendrium* do have practical value to the systematist, and the writer agrees with Yamaguti in their utilization. It is evident, however, that since *Acanthatrium* Faust,

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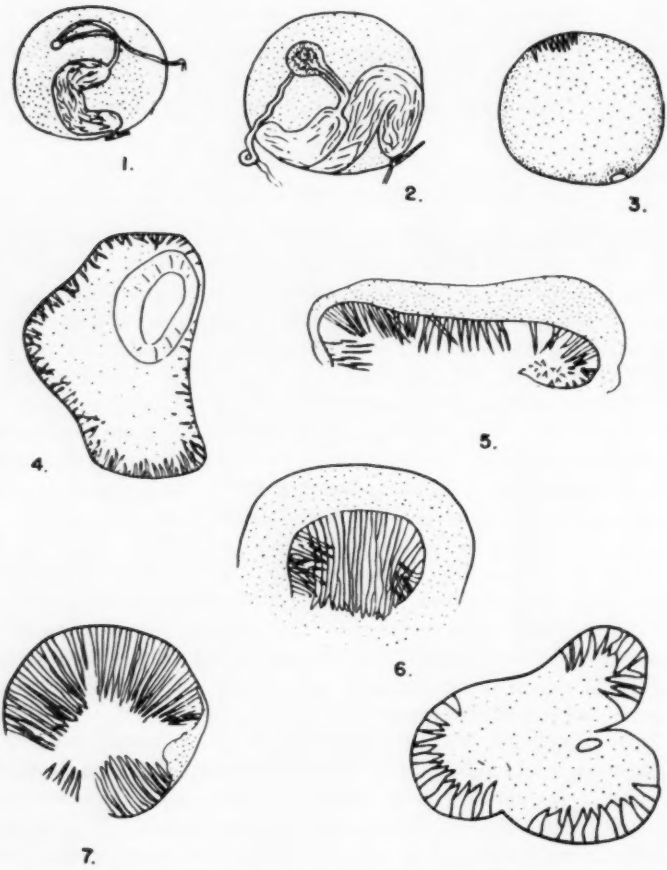

FIGURE 1. Prostate mass and seminal vesicle of *Acanthatrium* (*Prosthodendrium*) *swansoni*, after Macy (1936).

FIGURE 2. *A.* (*P.*) *naviculum*, after Macy, (1936).

FIGURE 3. Genital atrium of *A.* (*Acanthatrium*) *oligacanthum*, after Cheng (1957).

FIGURE 4. Genital atrium of *A.* (*A.*) *microcanthum*, after Macy (1940); atrium simple, with numerous small spines, 0.003 to 0.007 mm long.

FIGURE 5. Genital atrium of *A.* (*A.*) *oregonense*, after Macy (1939); atrium simple, with numerous long spines, 0.010 to 0.015 mm long, arranged in 3 groups.

FIGURE 6. Genital atrium of *A.* (*A.*) *pipistrelli*, after Macy (1940); atrium simple, 35 spines, 0.025 mm long, in 1 group.

FIGURE 7. Genital atrium of *A.* (*A.*) *eptesici*, after Macy (1940); atrium simple with numerous spines, 0.025 mm long, arranged in 3 groups.

FIGURE 8. Genital atrium of *A.* (*A.*) *alicatai*, after Macy (1940); described in text.

1919, has priority over *Prosthodendrium* Dollfus, 1931, Yamaguti's revision is untenable. Accordingly, the following species are transferred from *Prosthodendrium* to *Acanthatrium* in the subgenera indicated: *Acanthatrium* (*Prosthodendrium*) *anticum* (Stafford, 1905); *A.* (*P.*) *aranhai* (Lent, Teixeira de Freitas, and Proenca, 1945); *A.* (*P.*) *asadai* (Fukui and Ogata, 1941); *A.* (*P.*) *ascidia* (Van Beneden,

1873); *A.* (*P.*) *attenuatum* (Ogata, 1939); *A.* (*P.*) *bhaleraoi* (Pande, 1935); *A.* (*P.*) *brachycoelium* (Ogata, 1942); *A.* (*P.*) *buongerminii* (Lent, Teixeira de Freitas and Proenca, 1945); *A.* (*P.*) *chefrenianum* (Looss, 1896); *A.* (*P.*) *chilostomum* (Mehlis, 1831); *A.* (*P.*) *chosenicum* (Ogata, 1940); *A.* (*P.*) *cordiforme* (Braun, 1900); *A.* (*P.*) *diminutum* (Chandler, 1938); *A.* (*P.*) *dinanatum* (Bhalerao, 1926), type of the subgenus; *A.* (*P.*) *emollidum* (Caballero, 1943); *A.* (*P.*) *erhardovae* (Rysavy, 1954); *A.* (*P.*) *fukuii* (Ogata, 1939); *A.* (*P.*) *glandulosum* (Looss, 1896); *A.* (*P.*) *isikawai* (Ogata, 1939); *A.* (*P.*) *kitazawai* (Ogata, 1939); *A.* (*P.*) *kikugasirai* (Ogata, 1939); *A.* (*P.*) *labda* (Caballero, 1943); *A.* (*P.*) *lilliputianum* (Travassos, 1928); *A.* (*P.*) *longiforme* (Bhalerao, 1926); *A.* (*P.*) *lucifugi* (Macy, 1937); *A.* (*P.*) *luzonicum* (Tubangui, 1928); *A.* (*P.*) *macnabi* (Macy, 1936); *A.* (*P.*) *megacotyle* (Ogata, 1939); *A.* (*P.*) *mehrai* (Pande, 1935); *A.* (*P.*) *molenkampi* (Lie Kian, 1951); *A.* (*P.*) *naviculum* (Macy, 1936); *A.* (*P.*) *nokomis* (Macy, 1937); *A.* (*P.*) *oligolecithum* (Manter and Debus, 1945); *A.* (*P.*) *orospinosum* (Bhalerao, 1926); *A.* (*P.*) *ovimagnosum* (Bhalerao, 1926); *A.* (*P.*) *parvouterus* (Bhalerao, 1926); *A.* (*P.*) *posticum* (Stafford, 1905); *A.* (*P.*) *postacetabulum* (Yamaguti and Asada, 1942); *A.* (*P.*) *pyramidum* (Looss, 1896); *A.* (*P.*) *rhinolophi* (Ogata, 1939); *A.* (*P.*) *singularium* (Byrd and Macy, 1942); *A.* (*P.*) *skrjabini* (Shaldybin, 1948); *A.* (*P.*) *sphaerula* (Looss, 1896); *A.* (*P.*) *swansoni* (Macy, 1936); *A.* (*P.*) *tetralobulatum* (Caballero, 1943); *A.* (*P.*) *transversum* (Byrd and Macy, 1942); *A.* (*P.*) *urna* (Looss, 1907); *A.* (*P.*) *yamizense* (Ogata, 1939); *A.* (*P.*) *thomasi* (Sogandares-Bernal, 1956); *A.* (*Acanthatrium*) *alicatai* Macy, 1940; *A.* (*A.*) *amphidymum* Cheng, 1957; *A.* (*A.*) *anaplocami* (Etges, 1959); *A.* (*A.*) *eptesici* Alicata, 1932; *A.* (*A.*) *hitaense* Koga, 1953; *A.* (*A.*) *jonesi* Sogandares-Bernal, 1956; *A.* (*A.*) *macyi* Sogandares-Bernal, 1956; *A.* (*A.*) *microcanthum* Macy, 1940; *A.* (*A.*) *molossidis* Martin, 1934; *A.* (*A.*) *nycteridis* Faust, 1919, type of the genus and subgenus *Acanthatrium*; *A.* (*A.*) *oligacanthum* Cheng, 1957; *A.* (*A.*) *oregonense* Macy, 1940; *A.* (*A.*) *ovatum* Yamaguti, 1939; *A.* (*A.*) *pipistrelli* Macy, 1940. The following species are added to the subgenus *Acanthatrium*: *A.* (*A.*) *beuschleini* Cheng, 1959 and *A.* (*A.*) *sogandaresi* Coil and Kuntz, 1958.

The generic diagnosis given by Yamaguti (1958) for *Prosthodendrium* Dollfus, 1931 is equally valid for the present concept of the genus *Acanthatrium* Faust, 1919, and need not be repeated here. The type species for the genus is changed from *A.* (*P.*) *dinanatum* to *A.* (*A.*) *nycteridis*, as noted above.

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